PRACTICAL HISTOLOGY AND PATHOLOGY

HENEAGE GIBBES M.D.

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PRACTICAL HISTOLOGY AND PATHOLOGY

BY

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PREFACE TO THE SECOND EDITION.

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In this edition the whole work has been thoroughly revised and brought up to date. Several new chapters have been added. In Part II. the latest methods of cultivating and staining Bacteria are given.

I have again to thank Dr. Klein for his unvarying kind help, and also for allowing me to quote his most valuable article on the cultivation of Bacteria from the Local Government Board Reports. I have also to thank Dr. Arthur Ransome and Mr. Priestley Smith for kindly allowing me to insert some of their special work.

H. G.

94, Gower Street,
June 1st, 1883.

PREFACE TO THE FIRST EDITION.

—o—

THE object of this small work is to lay before the practitioner and student of medicine, a few concise and simple methods, by which the various tissues of the body may be prepared for examination with the microscope.

I do not claim any originality in these methods, but I recommend them from my own personal experience as the best, easiest, and cheapest to carry out.

The use of dyes for staining the tissues is a comparatively new branch of the science of Histology, and its value is being proved every day.

I have been engaged in experiments with all the various colouring agents for a long time, and in trying their effect in double and treble staining, and I have given the result of those which have proved most successful hitherto. I have also added a list of the aniline dyes, those soluble in water, and those soluble in spirit, which will be found very useful by any one wishing to make experiments in staining.

Briefly, but sincerely, I offer my best thanks to Dr. Klein, F.R.S., for the assistance which he has at all times freely and generously given me.

H. G.

94, GOWER STREET,
October 1, 1880.

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PART I.

PRACTICAL HISTOLOGY.

CHAPTER I.

INTRODUCTION.

HISTOLOGY, or the minute anatomy of healthy tissue as revealed by the microscope, has made vast strides in the last few years, and now forms a most important part of Medical education; a thorough knowledge of the normal structure of the animal tissues being absolutely necessary for the appreciation of pathological change.

This subject is now taught at the Medical schools, and there is no excuse for a student who has finished his curriculum without some knowledge of practical histology, and of the minute anatomy of the human body.

There are many, however, who qualified some years ago, when little attention was paid to this subject, who have consequently no practical knowledge of the methods required to prepare any morbid growth they may meet with in their professional career, or how to set about a microscopical examination of the same.

With a view to help those who have not been able to get the necessary knowledge during their student career,

and also those students who wish to form a Laboratory at home, the present work has been written, giving only the ordinary methods used by the author in his own Laboratory.

When all these methods have been thoroughly worked out, the student will find himself competent to try any of the various processes mentioned in larger works, and to judge of their utility.

Many men on reading the different hardening, cutting, staining, and mounting processes which any tissue has to undergo before it can be examined with the microscope, will be inclined to think it very tedious work. It is, however, a mere matter of routine, and when once this routine is established, the whole thing is comparatively simple. It takes very little time to change the hardening fluid, and if the student gets into the habit of looking over the bottles on the shelf every morning, where he keeps those tissues in the process of hardening, a glance at the labels will show those requiring a change. When the sections are mounted and examined under the microscope, he will find himself amply repaid for all his trouble if he has faithfully carried out the different processes in every detail.

It is always better to have one or two shelves devoted to those preparations which require changing; and those such as chromic acid, which require fresh fluid often, should be kept by themselves. Each bottle should be labelled, and the tissue, date, and hardening fluid, clearly written on the label. Every morning this shelf should be examined, and those requiring it changed; the date being each time written on the label, so that it may be seen at a glance how long the tissue has been in the fluid, and whether the hardening agent ought to be renewed. Müller's fluid, and bichromate of potash

preparations, may be placed by themselves, and need only be looked at occasionally.

A large outlay is not required for a course of Histological investigation, and the following list will show all those articles, re-agents, &c., which the student will find absolutely necessary.

1. Microscope. 1 Eye-piece. 2 Object glasses.
2. ½ gross ground edge, 3 × 1 slides.
3. 1 oz. No. 1, ¾ square cover glasses.
4. ½ oz. No. ⅞ ,, ,, ,,
5. 1 hollow ground razor.
6. Needles in handles.
7. 2 pair sharp pointed forceps.
8. 1 pair broad pointed forceps, not roughed, for taking up clean cover glasses.
9. Copper lifter.

RE-AGENTS.

¾ per cent. Salt solution.
½ ,, Solution Nitrate of Silver.
½ ,, ,, Chloride of Gold.
1 ,, ,, Carbonate of Soda.
Glycerine
Canada Balsam } In drop bottles.
A Bottle of Hollis' Glue.
A Williams' Microtome.
Watch Glasses.
Glass Capsules.
Dissecting Case.
Curved Scissors.

THE MICROSCOPE.

The most expensive as well as the most important article required is the microscope, and a good one should be procured.

In purchasing a microscope, it is necessary to go to an optician who makes his own instruments to get one that is worth anything, as a large number are made by wholesale manufacturers and sold to various traders who put their names on them and sell them as their own make. It is an easy matter to find out a *bonâ fide* maker, as there are very few of them, and it is their interest to sell a good instrument. A microscope such as that required by any one beginning a course of histology, can be procured for £5 5s., and nothing worth having can be bought at a lower price. If the instrument be procured from a *bonâ fide* maker it will last a lifetime with ordinary care.

The compound microscope consists of the stand, eyepiece, and object glasses.

Messrs. R. & J. Beck make a small microscope for £5 5s., with 1 inch and $\frac{1}{4}$ inch object glasses, and without coarse adjustment, which is quite good enough for all the ordinary work of a student. This stand will also do for all work usually required by a general practitioner, such as the examination of urine.

When, however, the student intends to do some scientific work in connection with his practice when qualified, he should get a rather more elaborate instrument.

For this purpose Messrs. Beck's Economic, with glass stage, is strongly recommended. The glass stage moving on a perfectly plane brass ring underneath gives a most

beautiful motion and does away with the necessity for a mechanical stage.

This stand, with a $\frac{1}{2}$ to $\frac{1}{6}$ and an achromatic condenser, costs £11 5s.

Messrs. James Swift & Son make a very neat student's stand at a low price, either with coarse adjustment or without. The stand without the coarse adjustment is quite enough for the ordinary work of a student, and with two powers costs £5 5. The body works in a cloth-lined tube, and with a little practice the student soon learns to bring the object glass nearly into focus before using the fine adjustment.

Mr. Crouch also makes a small instrument for students' use on a somewhat different model at the same price.

Zeiss's instruments are used by many in this country, and they are very good, but no one who has become accustomed to the movement of Messrs. Beck's glass stage will ever use any other. Zeiss's stands are rather more expensive than the English, his No. 2 costing about £12 10s., without eye-pieces or object glasses. He, however, makes another stand of the same size, without the revolving body, at a lower price. His stands are firm, but have the usual Continental flat foot, which is not so steady as the three points of the English instruments.

Having selected a maker, the student should get him to explain the working of the different parts, as all that is necessary for a beginner can be learnt in ten minutes in that way.

The student should ascertain by looking down the tube with the eye-piece removed whether the hole in the stage is concentric with the tube, and then try the different holes in the diaphragm in the same way.

The small student's stand is all that will be required

for a long time, as with it high powers can be used, and whatever work may be done in the future, it will always be the working stand.

The object Glass. The object glass or objective, is the most important part of the microscope, and it is necessary to have good glasses to do satisfactory work.

The most useful for the student are the ½ or $\frac{4}{10}$ for a low power, and the ¼, $\frac{1}{5}$, or $\frac{1}{6}$ for a high power; with a ½ and $\frac{1}{6}$ the student can do all the requisite work, and with the addition of an oil immersion $\frac{1}{12}$, he would be set up for life.

The student should get some good microscopist to test the object glasses for him before purchasing them, and he should see that they are tested on some histological object, and not on diatoms, as the wide angles necessary for revolving test Diatomaceæ are the reverse of useful to the young Histologist.

Object glasses ranging from 1 inch to $\frac{1}{6}$ can now be obtained at very reasonable prices. Those made by Messrs. R. & J. Beck, are very good and wonderfully cheap: a ¼ inch with perfect definition, costing only £1. Zeiss's glasses are also very good and can be procured from Baker, High Holborn. Messrs. Swift & Son, and Mr. Crouch also make some good powers for students' use. Glasses of a higher quality, having correction collars, are much more expensive, and are not really required for ordinary student's work; of these, Messrs. R. & J. Beck's $\frac{4}{10}$, $\frac{1}{5}$, and Zeiss's DD, ($\frac{1}{6}$), with correction, deserve special notice.

The most important glass for the student is the high power, and it is necessary that this should be a good one.

High Powers. The best high powers are made by Messrs. Powell & Lealand, they are of course expensive

but pay well in the end; they are made with correction collars, and are more adapted to large stands with mechanical stage.

They work close and require thin covers.

Any glass above a $\frac{1}{8}$ ought to be an immersion lens. Messrs. Powell & Lealand make some splendid glasses for water immersion, but the only one of these useful to a student of Histology is the $\frac{1}{12}$ on their new formula. This glass costs £13 13s., but is well worth it. Their higher powers work so very close that they are difficult to use.

Messrs. R. and J. Beck make some very good high powers, their $\frac{1}{10}$ water immersion is a splendid glass and very cheap; they also make a very good dry $\frac{1}{8}$.

Zeiss's E, which is a little lower in magnifying power than an English $\frac{1}{8}$, is also a very good glass.

Messrs. Beck's $\frac{1}{15}$, and Zeiss's J of about the same power are also very good glasses. Messrs. Beck make theirs either with or without correction collar, the latter a very cheap glass at £7, and invaluable where the student cannot afford an oil immersion.

These different objectives have been mentioned as they are useful in certain cases, but it is the opinion of some of the best microscopists of the present day, both in this country and abroad, that everything there is to be seen can be made out with a $\frac{1}{12}$, especially if it is an oil immersion. No great discovery has probably been made with a power above a $\frac{1}{6}$.

Oil Immersion Lenses. These glasses are taking the place of water immersion high powers in Histological research, as they have no correction for thickness of cover glass, and are consequently much easier to use; the only drawback is, that the essential oil used will dissolve Canada balsam, Dammar varnish, and many of

the other sealing fluids, and it is necessary to cover them with Hollis's glue which is not acted on by cedar oil.

These glasses were first made by Zeiss of Jena, and since by Messrs. Powell and Lealand. The first glass made by Zeiss was the $\frac{1}{8}$, he then brought out a $\frac{1}{12}$ and afterwards a $\frac{1}{18}$, of these the $\frac{1}{12}$ is unquestionably the best glass. Messrs. Powell and Lealand have made $\frac{1}{8}$, $\frac{1}{10}$, $\frac{1}{12}$, $\frac{1}{16}$, and $\frac{1}{25}$ on this principle, and have succeeded in removing a great objection to oil immersion lenses; they have made their glasses perfectly homogeneous, that is, they require no change of the oil when using oblique light on an object, and no correction with the draw tube is necessary when using a very thin cover. With Zeiss's $\frac{1}{12}$ it is necessary to draw out the tube two inches with a ·004 cover glass, and for central light a mixture of fennel and olive oils is required, which must be changed to cedar oil for oblique light.

With Messrs. Powell and Lealand's glasses cedar oil is used indifferently for central or oblique light, and no correction is necessary for a thick or thin cover glass.

Oblique light is of no use to the Histologist.

In using oil immersions, a very small drop of oil is placed on the front lens, the glass is screwed into the body, and lowered on to the slide until contact is made, which can be seen by bringing the eye to a level with the surface of the slide. The glass is then focussed by the fine adjustment until the object is seen sharply defined.

Oil immersion lenses are now being made with correction collars. That these are useful for very fine work there is no doubt. But with them the great advantage to a casual worker of getting the best effect the moment the glass is in focus is lost. It requires a great deal of practice to use the correction-collar, and a long time before the eye is sufficiently trained to recognize the

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exact point at which the object-glass is showing its best. Taking this into consideration it is certainly better for a man who is not going to work constantly with the microscope to get an oil immersion without a correction-collar.

When one of these glasses is in focus the immense superiority in brilliancy and definition over a dry glass* is at once seen. All slides intended for use with oil immersion lenses should be sealed with Hollis's glue as the cedar oil can then be wiped off without damage.

Eye-Pieces. Students' microscopes are generally sold with two eye-pieces, nos. 1 and 3, or A and C, but the no. 1 or A is the only one required, as no good result is obtained by using an eye-piece with a higher magnifying power, the higher eye-piece only magnifying the image seen with the object glass.

Higher eye-pieces are, however, useful in testing an object glass, as very many of those sold, while giving fairly good results with a no. 1 eye-piece, become blurred and indistinct when a no. 3 or 4 is used.

Illumination. Daylight is the best light to use for Histological work, and in the summer time there is generally enough light for ordinary work. In the winter, however, a lamp is often required even in the daytime, as there are many days when sufficient light cannot be obtained.

For ordinary work a common paraffin lamp with a flat wick, which can be purchased for 2s. 6d., is all that is required, more elaborate lamps can be got from the different opticians. The one thing requisite in a lamp is

* Dry glass means where the space between the front lens and the cover-glass is filled by the external air. Water immersion when a drop of water is placed between the two. Oil immersion when cedar oil is employed instead of water.

that the flame is steady, this depends on the wick fitting properly. When the flame flickers it is very trying and injurious to the eyes and should be remedied at once.

The illumination is one of the most important parts of microscopic work, and requires a great deal of practice before the best effects can be brought out.

For ordinary work with powers up to $\frac{1}{6}$ the mirror may be used, but with higher powers it is better to have the lamp sufficiently low to allow direct light to be thrown into the condenser.

When the whole field is to be examined the lamp is used with the whole breadth of the flame, but when a small portion is to be specially examined with a high power, it is necessary to turn the lamp so that the edge of the flame is presented, by which the light is very much intensified. The correct distance at which to place the lamp can only be found out by practice. A piece of blue glass should be interposed between the lamp and the condenser, this can be done by having it fitted into the condenser or by having a separate stand; different shades of blue will be found useful for various objects. The blue colour is a great help to the eyes and also throws up the stained specimen with more distinctness.

ACHROMATIC CONDENSER.

A small condenser made to go into the fitting under the stage will be found very useful, but for high power work with a large stand a wide angled condenser is necessary, with a graduated diaphragm by which the rays of light can be gradually cut off until the best definition is obtained.

Messrs. Powell and Lealand, and Messrs. R. and J.

Beck, make very wide angled condensers with double diaphragms, one carrying a series of holes to graduate the light, the other having blue glass of various shades.

To use the achromatic condenser it is necessary that it should be focussed on the object, this is done by moving it up or down until the lamp flame is seen sharply defined while the object is in focus; a bar of the window can be used in daylight. In the small stands the condenser fits into a tube under the stage and has to be moved up and down with the fingers. In the large stands it fits into the substage and is racked up and down. For very accurate work with high powers, Mr. E. M. Nelson has invented a fine adjustment for the substage.

STAND CONDENSER.

It is better to use the direct light of the lamp without the intervention of a stand condenser, as by this instrument the light is diffused and the sharpness of definition impaired. A stand condenser is required to throw a light on opaque preparations, but as these are not often used by the student of Histology, it is not required by a beginner.

ON LARGE STANDS FOR HIGH POWERS.

When very fine work, such as the measurement of objects under high powers, has to be done, a large stand is requisite having a mechanical stage and sub-stage, and it is important that the mechanism of the stand should be of the very best description. To get this it is necessary to go to the best makers and of course to pay a high price.

The best stands are made by Messrs. Powell and Lealand, and Messrs. R. and J. Beck.

The author has used a no. 2 stand of Messrs. Powell and Lealand for a long time, and found it all that could be desired.

ON THE BINOCULAR MICROSCOPE.

The binocular microscope has not met with much favour from Histologists as yet, from the fact that no power above a $\frac{1}{2}$ inch could be used without a special stand or apparatus costing a large sum. As, however, it has been shown by the author* that the binocular can be used with the $\frac{1}{12}$ oil immersion, the student should try it, and see what a different view it gives of various structures and their relation to one another. Any ordinary stand made on the Jackson-Lister model will do, so that the body can be brought almost into contact with the stage.

Messrs. R. and J. Beck have lately made a $\frac{1}{6}$ specially for the binocular which gives a very good result.

For a higher power Messrs. Powell and Lealand make their $\frac{1}{12}$ oil immersion with a screw cut on the outside, so that the front part containing the lens can be screwed into an adapter, which they supply with the glass. By this means perfect stereoscopic effect is obtained, and the observer is enabled to realize the precise relations of the different structures he is looking at to one another.

With Messrs. R. and J. Beck's new wide angled condenser and direct light from the lamp, both fields can be fully illuminated with Messrs. Powell and Lealand's $\frac{1}{16}$ oil immersion. It is stated by some eminent microscopists that the effect obtained is not stereoscopic

* See *Quarterly Journal of Microscopical Science* for July, 1880.

with such high powers unless a special set of prisms is used. This may be the case, but practically it is impossible to see that it is so, and by using the binocular in this way the eyes are saved, and the observer is enabled to tell if tracing a fine nerve fibre whether it passes over or under a cell or INTO IT, which appears to be all that is wanted, however wrong it may be theoretically.

CHAPTER II.

ON PREPARING TISSUES FOR EXAMINATION.

THE most essential point in microscopic investigation is the proper hardening of the material to be examined, and this must be done gradually, as if any tissue is placed in a strong solution, the elements of which it is composed at once shrink, and it is impossible to form a correct idea of their nature.

In making these hardening solutions French weights and measures will be found the most simple to use; but as many are not accustomed to them, the proportions will also be given in English weights when practicable.

One gramme is equivalent in weight to one cubic centimetre of water; it will therefore be seen at once how easy it is to make percentage solutions when French weights and measures are used.

For example to make a 1 per cent. solution, one gramme is weighed out and dissolved in 100 c.c. of water. The only apparatus required is a set of gramme weights and two measured glasses, one of 100 c.c. the other 500 c.c.

These can be procured from Griffin, Garrick Street, Covent Garden, or from Orme & Co., Barbican.

Hardening solutions as a rule do not require filtering.

The best plan is to make a large quantity at a time, say a Winchester quart. This holds about 2,400 c.c. of

water, this amount should be measured into the bottle, and the height of the fluid marked on the bottle with a diamond, the amount of the chemical used should be written on the label. So that when a new supply is wanted, the bottle has only to be held under the tap until the water reaches within a few inches of the mark, the amount of the hardening agent is then weighed out and put in the funnel, and the bottle filled up to the mark.

This does with substances that dissolve readily; others have to be pounded in a mortar with warm water.

1. *Chromic Acid Mixture.* The most useful hardening agent is a mixture of chromic acid and spirit.

Make a solution of chromic acid in water 15 grains to the pint, or 1 gramme to 600 c.c.; ordinary water may be used.

Take of this 2 parts, and ordinary methylated spirit 1 part—Stir.

The material must be cut into small pieces about half an inch square, and a large quantity of fluid used, a wide mouthed stoppered bottle holding from 6 to 10 ozs., according to the quantity of material, is best; change the fluid at the end of 24 hours, and again every third day, and the material will be hardened in from 8 to 12 days, this can be easily proved by taking out a piece and feeling it. If allowed to remain too long it gets brittle. When it is found to be moderately hard, usually after about 8 to 10 days, pour off the chromic acid mixture, and wash well, replace it by dilute spirit made thus:—

Take

Methylated Spirit 2 parts, and
Water 1 part.

Let the material remain in this for from 24 to 36 hours,

never longer than three days, and then replace it by pure methylated spirit; it may remain in this for an indefinite time, but it will often be found that the spirit becomes cloudy and full of deposits in a few days; in this case it is only necessary to change the spirit until it remains clear.

A large quantity of $\frac{1}{6}$ per cent. solution of chromic acid should be kept on hand, and it should be mixed with the spirit as required, it will be found the most useful of all the hardening agents, if it is changed at the proper time.

In some cases which will be mentioned further on, a $\frac{1}{6}$ per cent. solution of chromic acid may be used without the spirit with advantage. In other cases it may be necessary to use a solution much weaker, as a $\frac{1}{10}$ per cent. These are, however, not required for the ordinary purpose of the Histologist, but when special investigation is being made of some particular organ it is better to try the effect of different strengths of the hardening agent to see which gives the best result in that particular case.

2. *Müller's Fluid* is a good hardening mixture, but requires a much longer time, taking weeks to do what the chromic acid mixture will do in days. It is made thus :—

Take

Potass. Bichrom. 2 parts.
Sodæ Sulph. 1 part.
Water 100 parts.

In making this solution the ingredients should be pounded up in a mortar, and then warm water added until they are dissolved.

The advantage of this mixture is that larger pieces can be hardened in it, and it does not require changing

after the first week or two, but it will take from 5 to 7 weeks to harden anything, according to its size. The material, when sufficiently hardened, should be well washed and then placed in dilute spirit in the same manner as recommended after hardening in the chromic acid mixture.

3. *Dilute Spirits.* Many tissues can be hardened in spirit alone if they are placed in dilute spirit at first, so that the elements of which they are composed are not shrunk. This process is also used after hardening by any of the others.

Dilute spirit is made by adding 1 part of water to 2 parts of methylated spirit.

The material to be hardened should not be left in this mixture more than from 24 to 48 hours.

It is then transferred to pure methylated spirit.

4. *Bichromate of Potash.* Make a 2 per cent. solution and keep it on hand, as it is very useful for many tissues that require slow hardening. A solution can be made much more quickly with warm water than cold. This solution is also very useful to place portions of morbid material in, on their removal from the body in the post-mortem room, they can afterwards be transferred to the chromic acid mixture for more rapid hardening. This solution takes from three to seven weeks to harden, according to the size of the specimen, and the frequency with which the solution is changed.

5. *Bichromate of Ammonia.* A 2 per cent. solution is used in precisely the same manner as the former, and is applicable to the same tissues.

6. *Chromate of Ammonia.* Make a 5 per cent. solution, that is 1 oz. of the salt to 20 oz. of water, or 5 grammes to 100 c.c., and filter.

Keep it in a stoppered bottle. When this hardening agent is used for fresh tissue, such as mesentery, a small quantity is placed in a glass vessel and the tissue immersed in it for 24 hours, it is then washed until no more colour comes away and mounted in glycerine.

For other tissues it is necessary that the material should be cut into small pieces and left in the solution for 24 to 48 hours. It is then placed in distilled water, which must be changed several times until it is no longer tinged. The hardening is completed by the spirit process (page 17).

7. *Chloride of Gold.* Half per cent. solution. This is sold in small glass tubes, each containing 15 grains of the chloride, equal to 7 grains of pure gold. Take one of these tubes and file a ring round it above the bulb, it can then be easily divided into two parts, empty the gold chloride into a 6-ounce bottle, and wash out any particles that remain with distilled water, fill up the bottle. This will be under a half per cent. solution, but answers very well. Place a small quantity of this solution in a watch glass and immerse the tissue, which must be perfectly fresh, in it, let it remain in the dark for from half to one hour or more, then place in distilled water, which must be changed several times, and expose to diffuse daylight until it becomes a violet brown; about 24 hours will do in summer.

The tissue can then be mounted in glycerine, if it is a small thin substance, such as a tadpole's tail.

If, however, larger portions of any tissue are stained with gold chloride, the hardening will not be sufficient, and they must be further hardened by the spirit process (page 17).

Mouse-tail, stained and hardened by the gold process, may be decalcified by placing it in a half per cent. solu-

tion of chromic acid, or a saturated solution of picric acid for a few days.

The above process does very well for such small things as the tail of tadpole or mouse; but where portions of tissue are used certain modifications are required.

It is very difficult to get the gold to penetrate in the first place, and even more difficult to reduce it when this is done. For this purpose many processes have been tried. One of the best seems to be that adopted by Professor Ranvier.

He places the perfectly fresh tissue in lemon juice for five minutes before going into the gold solution. It may also be placed in lemon juice again after the gold, or in dilute acetic acid. But the best results so far have been obtained by placing the tissue in a solution of tartaric acid * until it is saturated, and then reducing by heat. Having used the lemon juice in the first place, the best plan to reduce the gold is to heat some water in a water-bath to a temperature of about 100° F. = 38 to 40 C., place the bottle containing the material in the tartaric acid solution, when it has become saturated, in the water-bath without a stopper, and let the two fluids stand at the same level. Keep at this temperature for ten minutes, and then allow it to cool gradually, wash well, and either cut sections at once or harden the tissue and cut in the usual manner.

The sections in either case must be exposed to daylight for two or three days in distilled water, which must be frequently changed.

Gold is a most provoking substance to work with, as it is not possible, seemingly, to depend on the result in any case, a brilliant specimen sometimes being made,

* The tartaric acid solution must be just under saturation.

which the same method will utterly fail to reproduce, although used in a precisely similar manner.

For another gold process, see Cornea. Gold chloride has a staining as well as a hardening action.

8. *Silver Nitrate.* Nitrate of silver possesses the property of forming a compound with the intercellular cement between the cells of serous membranes and other parts. For this purpose it is very useful for demonstrating the existence of squamous epithelium in such situations as the surface of the mesentery, or the lining of blood vessels. A ½ per cent. solution is used, made by dissolving 1 gramme of the salt in 200 c.c. of distilled water.

In the case of the mesentery, it is merely necessary to immerse it for two or three minutes in the solution, and then expose it to the light in distilled water for two or three days. It is necessary to change the water several times.

When the lining membrane of a blood vessel is to be demonstrated, or in any similar case where it would damage the part to remove it at once, the silver solution may be poured on it *in situ*, and after being allowed to remain for two or three minutes, washed off with distilled water, the tissue being afterwards dissected out.

In making specimens of mesentery it is necessary to be careful that they should be spread out without being stretched, which will destroy the silver lines.

The whole mesentery may be removed with the intestine and stained in silver, and then hardened in spirit. Some portions of mesentery will then be found stretched out in a natural manner, and these can be cut out and mounted in Canada balsam solution.

9. *Picric Acid.* A saturated solution of picric acid will decalcify small bones. It is also used in some

cases as a hardening agent by adding 1 part of water to 2 parts of a saturated solution; but it does not seem to give such good results as the chromic acid mixture.

10. *Osmic Acid.* This can be procured as a 1 per cent. solution in water, and it is then diluted to various strengths as required. It blackens fat and the medullary sheath of nerves.

A piece of mesentery placed in a weak solution for half an hour will show the fat cells lying along the course of the blood-vessels, as round black bodies.

It is also used for hardening the internal ear.

CHAPTER III.

ON CUTTING SECTIONS.

SECTIONS may be cut either by hand with a razor, or with a microtome.

1. In cutting sections by hand it is necessary to imbed the tissue in some material which will cut easily, and at the same time hold it firmly.

The best substance for this purpose is a mixture of wax and olive oil.

Take equal parts of white wax and olive oil by weight and melt them together, pour into a shallow vessel and when cold cut into small blocks.

Small tin boxes with a removable bottom are required to hold the mixture while the tissue is being imbedded, and the best size is 2 inches long, by $\frac{3}{4}$ of an inch wide, and $\frac{3}{4}$ of an inch deep.

It will be necessary also to have a small porcelain ladle and a stand to raise it above a spirit lamp or gas jet. Melt some of the wax mass in the ladle, and be careful not to make it too hot.

Prepare the tissue so that the face which is to be cut can be easily recognized, stick a needle into it away from the part which is to be cut, drain off most of the spirit by laying it on filtering paper, and then immerse in the melted wax mass, so that it is perfectly covered, take it out and let it cool. Take a small piece of filter

paper and place it over the removable bottom of the tin box, and then fix it in its place, the filter paper will prevent the wax from running out if the bottom fits loosely. It is also useful to leave a little of the paper projecting on which to write the name of the material imbedded. Then half fill the box with melted wax mass and hold the material in it, keeping it quite steady until the wax hardens, then by gently screwing the needle round it can easily be removed, and the box filled up with wax mass. It will be found a saving of time to imbed a portion of material at each end of the box. When the wax mass has become thoroughly hard, which will take some time, especially in warm weather, pull off the bottom and push the wax mass, with the filter paper adhering, out of the box. It can then be laid by until wanted, the name of the material imbedded being written on the paper.

For cutting sections thus imbedded, a hollow ground razor is necessary ; a very good one for this purpose can be procured from Baker, High Holborn ; the razor must be very sharp. A small glass capsule about ¾ of an inch deep filled with methylated spirit is also required to put the sections into when cut, and to moisten the razor in.

Take the wax mass and with a scalpel carefully remove small slices from one end, until the imbedded mass can just be seen, then take the razor and dip it into the capsule, taking up a little spirit, let this run along the edge so as to thoroughly moisten it, and commence cutting as thin sections as possible, by drawing the razor diagonally across the mass with a steady sweep; this must not be done too quickly, and the amount of pressure to be put on the razor will depend on the tissue imbedded and can only be learnt by prac-

tice. As each section is cut dip the razor into the capsule of spirit and wash it off. Wipe the razor occasionally and remove adhering portions of wax mass, and always keep the edge wetted with spirit. When a sufficient number of sections have been cut, the thinnest should be selected and removed to a watch-glass containing clean spirit.

Great care is required in cutting sections by hand, to hold the razor firmly yet lightly, so as to cut them thin and at the same time even, and this cannot be done without a great deal of practice.

For larger sections the boxes must be proportionately increased in size, and it will be found convenient when the wax mass is as wide or wider than the razor to cut off slices from each side so as to reduce the surface to be cut as much as possible without interfering with the stability of the imbedded material.

A small flat spear-headed needle will be found useful for taking up very small sections.

THE FREEZING MICROTOME.

A much easier method of cutting sections is by using a microtome. Of these there are a number made, in some of which the material is imbedded in wax mass, or a mixture of paraffin and lard, and raised gradually by a screw, while a razor is worked on a flat plate shaving off sections; these are also made with a chamber to contain a freezing mixture, so that the material can be imbedded and frozen.

The best microtome for histological use, is the Williams, made by Messrs. Swift and Son of Tottenham Court Road.

It consists of a tub to contain the freezing mixture,

with a brass standard into which screw the brass circular plates on which the material is frozen. A top with a glass surface fits on to this, having a hole through which the circular plate projects. The knife is fixed into a triangular frame, having screws at each angle by which it is raised and lowered.

To prepare the microtome for use :

1. Have the knife as sharp as possible.
2. Pound some ice finely in a cloth.
3. Scrape some salt into a fine powder.

With a spoon, put a layer of ice into the tub and then some salt on it, mix with the spoon, and so on, until the tub is about half full, then ram it hard with a stick and fill again, put in the salt and ice in about equal proportions, leave room for the top, wipe off the salt and ice from the edge, put on the top and fix it with the screw for that purpose. Screw the circular plate into its place, and the microtome is ready for use.

An India-rubber tube must be fixed to carry away the drainings as the ice melts.

Mr. Groves of King's College has adapted an ether spray to this machine, by which it can be used at any moment without the trouble of procuring ice. This instrument will be found a great convenience to those who only do occasional work. The ether fumes are carried away, by a waste tube, which can be led outside a window so that no unpleasant smell is developed in the room.

Dr. Roy has invented a microtome on a different principle, but which is used with the ether spray. It is made by the Cambridge Scientific Instrument Company. It is invaluable, when fresh material has to be examined, as a small portion is taken, laid on the brass plate, covered with mucilage, and frozen at once. This can

be done more quickly than with either of the other instruments.

TO PREPARE THE MATERIAL FOR FREEZING.

Any tissue which has been preserved in spirit, must be soaked in water for about 12 hours to remove the spirit, and then placed in mucilage for about 6 hours.

It will be found a great saving of time, when a number of specimens are to be cut at one freezing, not to have the material too thick, as a piece a quarter of an inch thick will give an enormous number of thin sections, and take only a short time to freeze.

TO MAKE MUCILAGE.

Pour warm water on picked gum Acacia and make a solution rather thinner than the mucilage sold in the shops.

CUTTING THE SECTIONS.

Remove all the specimens to be cut, from the gum, and place them in a small saucer ready at hand. Take up one with a pair of forceps, and lay it on the circular plate of the microtome, drop some gum solution on it with a small brush, and see that it runs down on to the plate all round the specimen so as to fix it firmly. When it is thoroughly frozen adjust the razor so that it will just pass over without touching. Then lower a little more and try each end of the razor against the frozen material to see if it is quite level. If one end is too low, raise it until they both make the same cut.

Raise the frame again until it just clears the material, and then lower the screw at the apex each time, by giving it a slight turn to the left, so that the knife takes off a thin section of the frozen material. The knife must be pushed across in a diagonal direction.

Very slight pressure must be made from above, the pressure necessary to carry the knife through the material, is made by the two thumbs, applied to the base of the brass frame. The student should accustom himself to hold the frame with the fingers of the left hand in such a manner, that he has a firm grasp of it, and can take off the sections with a brush, and not drop the razor and frame. Have a small vessel ready, containing warm *distilled water.* It is necessary to use distilled water, as in ordinary water the lime in solution is precipitated by boiling and the specimen will be covered by fine particles of carbonate of lime and utterly ruined. Moisten the upper surface of the razor with gum solution, and the sections as they are cut will slip up on it without curling, carefully remove them with a camel-hair brush and place them in the warm distilled water and let them remain for 10 minutes or longer, until the gum is dissolved out; this will take longer with some material, such as testicle, than others.

With these microtomes the most beautiful sections can be cut, perfectly even throughout, surpassing anything that can be done with the hand. With care very large sections may be cut quite as readily as smaller ones, but the razor must be very sharp, and the material not too hard ; those hardened in chromic acid mixture, No. 1 (page 15) seem to do the best. Very little force is required in pushing the knife across the material, and if it is sharp a very slight turn of the screw each time

will cut a section ; these ought to be so thin as to be almost invisible, as the gum melts on the razor.

In cutting some material, such as retina, it is advisable to stain it *en masse* before freezing, otherwise the sections cannot be seen when placed in water.

CHAPTER IV.

ON STAINING.

List of staining agents :—

1. Hæmatoxylin or logwood.
2. Picro-carmine.
3. Eosin.
4. Soluble Aniline Blue.
5. Gentian Blue.
6. Methyl Blue.
7. Iodine Green.
8. Methyl Green.
9. Rosanilin Hydrochloride.
10. Rosanilin Acetate.
11. Safranine.
12. Vesuvin.
13. Spillers Purple.
14. Osmic Acid.
15. Chloride of Gold. } Selective Stains.
16. Nitrate of Silver.

To demonstrate the minute structure of any tissue, the sections require to be stained with some colouring agent which will show the different elements more plainly by their absorption of the colouring matter and bring out very transparent parts which otherwise would be hardly discernible.

A large number of staining agents are not required. Those enumerated in the list will be amply sufficient for the Histologist and Pathologist. This is the result of a number of years' experience in which every stain has been tried. The enormous number of Aniline dyes now made are the result of the different manufacturers giving different names to the same compound, or to those which differ so slightly as to be of no practical value. The great desideratum is to get stains that are really what they profess to be; Messrs. Hopkins and Williams are to be thoroughly relied on, and the crystals can be obtained from them in small quantities. Messrs. R. and J. Beck also sell the solutions made up ready for use as well as the crystals.

LOGWOOD STAIN.

1. Take of

Extr. Hæmatoxyl grms. 6
Alumen grms. 18.

Mix thoroughly, while mixing add 28 c.c. of distilled water. Filter and add to the filtrate ʒj of spirit of wine. Keep in a stoppered bottle a week before using. What remains on the filter can be mixed with 14 c.c. of distilled water, and left soaking in it for an hour or so, then filter and add to the filtrate ʒss of spirit of wine. This second solution is as strong as the first.

The alum used must be potash without ammonia, and the extract of logwood must be English, the best is sold by Messrs. Hopkins and Williams, Cross Street, Hatton Garden.

TO STAIN WITH LOGWOOD.

Make a cone with a small round filter paper, and pour some of the staining fluid into it, let from seven to ten drops fall into a watch glass and dilute with *distilled water*. Let the sections remain in the solution for about a quarter of an hour, the time will depend on the tissue and the manner in which it has been hardened. Some tissues take in the stain very rapidly, others slowly. Take out a section from time to time, and place it in a watch glass of ordinary filtered water to see if it is stained deeply enough.

When the sections appear to have stained thoroughly remove them to a watch glass of ordinary water, and wash them to remove the excess of colouring matter. In staining with logwood it is necessary to be careful that too many sections are not placed in the solution at once, as they will lie thickly one on the other, and the staining will not be uniform; it is also necessary to dilute the logwood stain with distilled water, as ordinary water will not give the same result, owing to the different matters held in solution; but it is better to use ordinary water for washing the sections after staining as it helps to fix the colour.

The solution should not be too strong, as better results are obtained from staining the sections slowly than from doing it rapidly, and it will always result in loss of time if an attempt is made to stain a large number of sections in a strong solution.

ON STAINING SECTIONS WITH LOGWOOD THAT HAVE BEEN PREPARED WITH CHROMIC ACID.

It is necessary to remove the chromic acid from sections hardened in that fluid, and this is done by taking some of the 1 per cent. solution of Carbonate of soda, and soaking the sections in it for some time; ten to twenty minutes will be sufficient.

Then remove them to plain water and wash well.

Prepare two watch glasses of dilute logwood stain and place the sections in one of them, let them remain for a minute and then place them in the other; there they must stay until stained deeply enough.

If the first watch glass be now examined, the logwood stain will be found to have become quite granular, and if this precaution had not been taken it would have been deposited as minute granules all over the sections.

2. *Picro-carmine.* The following method of Professor Ranvier is the best for this stain.

Rub up 1 gramme of carmine with 10 c.c. of distilled water, add 3 c.c. of Liq. ammonia.

Add to this, 200 c.c. of a cold saturated solution of picric acid.

Evaporate slowly in a water bath to one third at a low temperature.

To use the stain filter about 10 drops into a watch glass and dilute with distilled water. The sections must remain for some time, from twenty minutes to half an hour, and if at the end of that time they have not stained sufficiently, a little more picro-carmine may be added.

They are then placed in water acidulated with a few drops of acetic or picric acid and left for an hour.

When making experiments in treble staining a number of sections may be stained in picro-carmine and then placed in methylated spirit; there they may remain until required, as the spirit does not affect the stain, which forms a very good ground colour, on which to try combinations of different anilines.

It is also a good stain for fresh tissues, such as mesentery when used with logwood. Also in sections of skin it is very useful as a ground colour, and by staining a number of sections of the same tissue with picro-carmine and then with two other colours, a variety of results will be obtained.

In some the tissue for which picro-carmine has a special affinity, such as connective tissue, will be found unaltered, while the surrounding tissue has taken on the new colours. In others the picro-carmine has combined with one or both, and a new colour is formed. Or again, it may be entirely supplanted by one of the other colours.

3. *Eosin* is not an anilin colour but is a potash salt of resorcin. Resorcin is obtained by the action of melting potash on galbanum. Eosin is largely soluble in water and has a beautiful garnet red colour. In using it a strong solution is required and the sections must be well washed in water after staining. It is a very delicate colour when used alone, and almost too transparent, but in combination it becomes opaque.

To make this stain, 5 grammes of the powder should be rubbed up in a glass mortar, with 100 c.c. of distilled water, making a 5 per cent. solution.

4. *Soluble Anilin Blue.* This is a useful stain for some tissues, such as stomach and spinal cord. It is very easily made but requires to be strong, nearly saturated, some of the granules should be rubbed

up in a mortar until quite dissolved, and some rectified spirit added, about 10 c.c. of spirit to 100 c.c. of water, as it is very apt to grow mould on the surface.

Sections must be deeply stained, as a good deal of the colour comes out in the spirit. They should be only lightly washed in methylated spirit and then transferred to absolute alcohol, which does not affect the colour so much. There are several soluble Blues sold, but that of Messrs. Hopkin and Williams is the only reliable one.

5. *Gentian Blue.* This is a most useful colour for Bacteria, but it must be genuine. That sold by Messrs. Hopkin and Williams was originally procured for the author, and is the only one that will give the desired result. A $\frac{1}{2}$ per cent. solution is the best strength to use. The application of this stain is given under the heading Bacteria.

6. *Methyl Blue.* This stain is used as a contrast or ground stain for the Tubercle Bacillus, and the process will be given under that heading. A strong, nearly saturated, solution is required; a good proportion of rectified spirit must be added to make it keep—one drachm to the ounce.

7. *Iodine Green.* A dark green, and very durable, standing spirit well and not fading. It is an invaluable colouring agent in double staining, as it is not so opaque as anilin blue. Make a 5 per cent. solution in water and filter, place a few drops in a watch-glass and dilute; it is very strong, and the section, when taken from spirit, will float on the surface, where it may be seen taking in the colour; if a light stain only is required it will be sufficient to let it remain on the surface; but if a darker stain is wanted it must be wholly immersed, and then it must not be left long or the stain will be too deep, and it cannot be afterwards removed.

This is one of the most useful of the anilin colours, and the results, when it is carefully used, are very beautiful; it picks out all the nuclei, and in growing bone it colours the unabsorbed cartilage, giving a very striking result. It is also a most valuable anilin in double staining; its action will be described under that head.

8. *Methyl Green.* This is a lighter green than the Iodine, and is used in the same manner. A 2 per cent. solution is usually strong enough. This stain has a peculiar affinity for the heads of spermatozoa.

9. *Rosanilin Hydrochloride.* This stain is useful for double and treble staining; for this purpose a strong solution must be made in rectified spirit. Place some of the crystals in a glass mortar and rub up with a little spirit, add more spirit, until all the crystals are dissolved. This will do for the ordinary staining processes. It is also used in a special manner for the Tubercle Bacillus, and the method of making the stain will be given under that head.

10. *Rosanilin Acetate.* This is a useful stain for blood corpuscles. It must be rubbed up in a mortar with rectified spirit, and when thoroughly dissolved an equal quantity of distilled water added—5 grammes of the crystals to 100 c.c. of spirit and thoroughly dissolved; then 100 c.c. of distilled water added—this makes a good strength for general use.

11. *Safranine.* This colour is useful for the detection of amyloid degeneration. A 1 per cent. solution is made with distilled water and filtered. The sections must be left in the stain for half an hour and then washed well in water. They must then be placed in methylated spirit and washed until the colour comes away very slowly. A little practice is required to do this properly, as if they are left too long all the colour will come out, and if

they are not washed enough in the spirit they have an opaque blurred appearance.

12. *Vesuvin and Chrysoidin.* These stains are used in aqueous solutions. Vesuvin requires to be made very strong. A 10 per cent. solution may be made and diluted. It is a very pleasant stain for the eye and differentiates fairly well.

Chrysoidin is used for a ground stain with sputum, containing Tubercle Bacilli. It is of no use for tissues as it fades very quickly on exposure to light.

13. *Spiller's Purple.* This is a very useful colour for double staining and also for Bacteria. For double staining a 2 per cent. solution in water is required, and the section must be very deeply stained as a great deal of the colour is washed out by the spirit. For Bacteria a 1 per cent. solution is the best made with distilled water.

Gold Chloride, Silver Nitrate, Osmic Acid. These selective stains, having a hardening as well as staining action, have been considered under the head of hardening agents. See pp. 18, 20, 21.*

* Solutions of these stains can be obtained ready for use, from R. & J. Beck, 68, Cornhill.

CHAPTER V.

DOUBLE STAINING.

By double staining is meant a process in which two colours are taken, which have affinities for different elements in the tissues to which they are applied. Thus while one colour will stain the connective tissue and protoplasm of cells, the other will colour all nuclei and so differentiate the different elements as to make them more easily discernible. Others again will stain different glands according to their secretions. Thus showing a distinct chemical reaction between glands differing in their functions.

In other cases the duct of a gland can be stained of a different colour to the surrounding tissue and its own secreting substance, by which means it is easy to distinguish it, and thus show if it is implicated in any morbid change, and also in some cases prove whether the morbid change is primary in it, or has extended from surrounding tissues, in which case all the ducts would not probably be similarly affected.

Double staining is a subject that requires to be very much more worked out than it has been hitherto, and in the present work, those processes only will be given in detail, which have been fairly well tried; many other combinations of staining agents will be apparent to the student, which have not yet been worked out, for want

of time, and there is no pursuit in which patience and time for experimenting are more required, than in double staining.

The student must not be discouraged by many failures, as there is always some new fact to be learnt and noted, and in the application of these to future experiments some brilliant results are sure to be obtained.

PICRO-CARMINE AND LOGWOOD.

This combination has been used for a long time, and gives very satisfactory results. The student should begin with sections of scalp, skin, or tongue, and the result, if the process be carefully carried out, will be found very satisfactory.

The sections must be first stained in picro-carmine and then in logwood. Make a dilute solution of picro-carmine in distilled water, about 10 drops to the watch-glass, and let the sections remain in it for from twenty minutes to half an hour, then wash in water and place in distilled water acidulated with 1 or 2 drops of acetic or picric acid. Let them remain in this for about an hour. Remove the sections from the acidulated water and place them in dilute logwood stain; this should not be too strong, from 5 to 7 drops to the watch-glass of distilled water; do not let them stain too deeply. When sufficiently coloured, which will be shown by their becoming a faint lilac colour, they must be washed to remove the excess of logwood, and mounted in the usual manner.

This double stain is very effective when used with fresh tissues, such as serous membranes; it brings out the connective tissue corpuscles in the mesentery of the

newt, and at the same time shows the non-striped muscle tissue very well. It is also useful in bringing out the delicate tissue in the tubuli seminiferi of the testis and showing the developing spermatozoa there.

In any tissue where there are elements of different kinds, such as scalp or developing bone, it will be found to give very good results. The logwood stain must not be too deep, as it is a very opaque colour.

PICRO-CARMINE AND ANILIN COLOURS.

Some very good results may be obtained by staining sections first in picro-carmine, then letting them remain in acidulated water for an hour, and afterwards staining them with various solutions of anilin colours.

Safranine, after picro-carmine, gives a good double stain, as the picro-carmine colours all the connective tissue and nuclei, while the safranine stains muscle, epithelium, &c.; but the two colours are not sufficiently different to give as good a result as logwood and picro-carmine, although they will be found useful where great transparency is desired.

Picro-carmine and iodine green give a very beautiful effect when it is wanted to isolate gland tissue; such as Peyer's patches, or the glands in the tongue, œsophagus, or solitary glands in the large intestine. The picro-carmine staining everything but the glands, which remain a bright green.

Eosin and anilin blue give good results, but require to be used cautiously, as if the staining is too deep the section becomes opaque. To get the best effect, the section should be very thin, and must be well washed after staining with eosin, and then just immersed for a few seconds in the anilin blue.

A great many other combinations will suggest themselves to the student, and he will be amply rewarded by experimenting further with the various staining agents mentioned.

TREBLE STAINING.

The combination which has given the best effect so far in treble staining is: picro-carmine, rosanilin, and iodine green. Stain the sections well according to the process already described for picro-carmine, and soak them in acidulated water. Then take a few drops of the solution of hydrochloride of rosanilin, No. 9, dilute it with spirit, and immerse the sections for two or three minutes, remove them to methylated spirit and wash off the excess of colouring matter. Then place them in a dilute solution of iodine green. Coming from spirit they will float on the top of the watery solution, and this in many cases, when the green stain is not required to be very deep, is quite sufficient. When a deeper stain is required, immerse them altogether, and let them remain a minute or two; but it must be borne in mind that this colour cannot be washed out again if too deep, which the spirituous stain can, so that it is better to have a section apparently over-stained in the rosanilin solution, while it is even under-stained in the iodine green. After washing, the sections are mounted in the usual manner. It will be found, however, that a good deal of the rosanilin will come out in the second immersion in spirit, and it is necessary to change it until no more colour comes away; otherwise the oil of cloves will become coloured, and from it the Canada balsam, in which the specimen is mounted.

With the above mentioned three colours, the most

beautiful effect may be obtained, but it will take some time and practice to get the process exactly right, and this is a matter which can only be gained by experience. The results will be found to vary with the length of time the section is immersed in each of the two last colours, and also with the strength of the solutions.

The sections should be uniformly and deeply stained with picro-carmine. The other two solutions should be saturated in the first instance, and then diluted one-half at least. If they are to be laid aside for some time before mounting they should not be left in spirit, but in oil of cloves. Only a few sections should be stained at one time or some will be found much more deeply stained than others. The best results will also be obtained with material that has been hardened in chromic acid.

The staining process is well shown in a section of the base of a cat or dog's tongue, cut through one of the circumvallate papillæ; the section should be sufficiently large to include some of the mucous glands, of which there are a large number in that region.

If the staining is well done it will show all the muscle fibres stained with picro-carmine, the connective tissue, protoplasm of cells, &c., stained red; while all the nuclei in the superficial epithelium, serous glands, non-striped muscle tissue in the vessels, and elsewhere, are stained a brilliant green.

The most important fact demonstrated by this process is the different chemical reaction shown by the various glands. In the mucous glands, while the epithelium lining the duct is stained in precisely the same manner as the superficial epithelium of the organ, it will be found that the moment the secreting epithelium is reached a new colour presents itself, which differs *in*

toto from either of those employed in the process; thus showing that the secretion has the power of causing these two colours, green and red, to combine, forming different shades from purple to blue,* according to which colour predominates. In the serous glands, however, quite another aspect is presented; there is no combination as in the mucous glands, but the protoplasm of the cells is stained more or less deeply with red, while the nuclei have taken on the green; the colour differs, however, from that of the surface epithelium, and appears to have taken on picro-carmine to some extent, which, with the rosanilin hydrochloride, gives a dull red colour.

In many places will also be seen small masses of adenoid tissue which have stained a bright green throughout.

Altogether this makes one of the most brilliant specimens in the whole range of histology, and although the process is rather troublesome, and requires a certain amount of practice to determine the time required for each immersion, it amply repays when once properly done.

Take only a few sections at a time, and do not hurry over the different processes, and after a few trials the exact time of immersion will be hit on, and should be recorded.

CHLORIDE OF GOLD AND ANILINES.

Some very striking results may be obtained by first staining fresh tissues, especially growing bone, in chloride of gold solution (page 18), and then decalcifying and hardening in spirit. After the material has har-

* Iodine green is a very blue green.

dened sufficiently, sections may be made and stained with two colours. It is not quite clear what action the gold chloride has on those parts it does not stain, but that it has some, is evident from the difference of the action of anilin dyes on those specimens prepared in gold, from those hardened in any other manner.

A very good material for the purpose is the tail of a young rat or mouse, placed in half per cent. solution of gold chloride for an hour or two, and then decalcified and hardened in the usual way. Very thin transverse sections should be cut, and stained first in rosanilin and then in iodine green.

On examining the specimen the gold staining will be seen in the periphery, bringing out the tendon cells, and giving a dark hue to everything for a certain distance from the outside ; but within this a great variety of colour will be found, the different tissues being stained in a most gorgeous manner. In the middle the bone trabeculæ will be seen faintly stained, while the calcified cartilage, in their centres, is stained a bright colour, totally different. All these colours may be varied by using different anilin solutions, and a very pretty result may be obtained by simply staining with iodine green.* In the above instance the true bone is only faintly stained, while the calcified cartilage takes the colour deeply.

* Methyl green does just as well.

CHAPTER VI.

ON MOUNTING.

SLIDES.

GLASS slides, 3 × 1, must be cleaned before using, and a good plan is to keep some cleaned, ready for use, in a two-dozen box with rack work, where they stand on their edges and do not get dusty. The ordinary slides sold at the shops at 6s. a gross, are easily cleaned with a chamois leather. Sometimes, however, especially when using slides for the second time, they cannot be cleaned so readily, and they must be soaked in a decoction of oak galls for some hours; this is made by pouring boiling water on bruised oak galls and straining.

COVER GLASSES.

The usual size of these is $\frac{3}{4}$ of an inch square, but larger ones will be required, and some of $\frac{7}{8}$ of an inch should be obtained.

For ordinary work square cover glasses will do, but for objects that have to be sealed up with Hollis's glue, either because they are to be examined at once with an oil immersion or when they are mounted in a watery medium or glycerine, round covers are better, as they can be so easily sealed with a turntable. They must,

however, be mounted in the centre of the slide, and for this purpose what is called a mounting card is used. This is merely a square piece of cardboard with a $\frac{3}{4}$ inch circle and centre dot, while two pieces of cardboard at right angles are glued on to it, so that when the slide is pushed up against them the circle and dot show through where the exact centre is.

The student is cautioned against buying cheap slides and cover glasses as they do not pay, a large number having to be rejected on account of flaws, it is much better to pay a little more and get slides which will not spoil a good preparation.

ON MEASURING COVER GLASSES.

For ordinary students' work the No. 1 cover glasses will do perfectly well, as they are very near the thickness to which object glasses, without a correction collar, are adjusted; but when high power glasses are to be used, it facilitates the work very much, to know the exact thickness of the cover glass under which the specimen is mounted, and with very high powers, or those with wide angles of aperture, the cover must be at least ·004 to enable the glass to work through it. Powell and Lealand's $\frac{1}{25}$ water immersion requires a cover glass ·003 of an inch.

It is a good plan to measure an ounce of No. 1 cover glasses occasionally, and put by those of thicknesses of ·004 and under. This is readily done by a small lever instrument made by Messrs. Stevens & Sons, instrument makers, 159, Gower Street, only a few of these very thin glasses will be required for special work, but it is as well to have them in readiness.

ON CLEANING COVER GLASSES.

The following plan will be found a very good one, both for saving time and breakage.

Place the cover glasses to be cleaned in a glass vessel containing strong sulphuric acid, and agitate gently until the acid has penetrated between the glasses and driven out the air-bubbles, let them remain in this for an hour or two and then wash well in water until no acid is left. Remove them to a capsule containing methylated spirit. Take out each one separately with a pair of broad pointed forceps, and wipe dry with a silk or soft linen rag.

With very thin cover glasses, such as ·003, each glass may be dipped in absolute alcohol when taken out of the methylated spirit and then carefully dried with an old silk handkerchief.

MOUNTING FLUIDS.

For fresh tissues :

Glycerine.

For hardened tissues :

Canada balsam solution.
Dammar varnish.

MOUNTING FRESH TISSUES.

Place the tissue to be mounted in a capsule of water of sufficient depth to cover more than half of an ordinary glass slide, when placed in it with one end on the bottom and the other resting on the opposite side.

With a needle, bring the tissue over the middle of the slide and hold it there, at the same time raise the upper end of the slide very gently, so that the tissue will adhere to it and be raised out of the water. See that it is not folded in any part. Lay the slide on some filter paper, and with needles spread out the tissue to its fullest extent, without stretching it. It is necessary to be very careful of this, as if the tissue be a serous membrane, stained with silver, the outlines of the cells will be completely destroyed wherever it has been stretched. In the same way, non-striped muscle fibre in the mesentery of the newt, will be broken up and quite ruined.

When the tissue appears to be extended in a natural manner, without folds, take up the slide and wipe off all moisture from it with a clean cloth. If there is a large quantity on the specimen, some may be removed with a bit of filter paper, but great care must be taken not to touch the specimen itself with the paper as it will adhere to it; at the same time it must not be allowed to become dry, and if this seems probable, it can easily be moistened by breathing on it occasionally, until the cover glass is ready. Take up a clean cover glass and place a drop of glycerine on the centre, invert and place it horizontally on the specimen, leaving the weight of the cover glass to spread out the glycerine. If there is an excess of glycerine round the edges of the cover glass, it must be removed by placing small pieces of filter paper in contact, which will soon absorb the superfluous fluid, but must not be left too long or they will drain it from under the cover glass. When the superfluous glycerine has been removed by the pieces of filter paper, take them off and wipe the slide with a dry cloth, taking care not to move the cover glass.

When this is done the preparation must be sealed, by painting round the cover glass with either Dammar varnish or Hollis's glue, taking care that only the extreme edge of the cover glass is included. It will be necessary to give a second and third coat if Dammar varnish is used, at intervals of a few days.

It will be found a good plan to seal first with Dammar varnish, and afterwards to cover this with Hollis's glue, as it makes the preparation more secure, and it is absolutely necessary to have them sealed with Hollis's glue when oil immersion lenses are to be used, as the cedar oil does not touch it, while it dissolves Dammar varnish at once.

Specimens carefully prepared in the above manner may be kept for years without deteriorating.

MOUNTING IN CANADA BALSAM OR DAMMAR.

Canada balsam mounting fluid is prepared by mixing:

Canada balsam, 105 parts, or 3 ozs.
Turpentine, 35 parts, or 1 oz.
Chloroform, 35 parts, or 1 oz.

Dammar varnish is prepared thus :
Take of

Gum Dammar in powder, ½ oz., and dissolve it in turpentine, 1½ oz. Filter.
Gum mastic, ½ oz., and dissolve it in chloroform, 2 ozs. Filter.

Mix the two solutions and filter again.

Put in stoppered bottles, and see that they are perfectly free from moisture before using. A small drop-bottle of each of these fluids must be kept for daily

use, and when they get thick from evaporation a little chloroform can be added.

Both of these mounting fluids are used in the same manner, and one description will apply equally well to each. Canada balsam is the one commonly used, as the materials of which it is composed are very cheap, while gum dammar is rather expensive. The dammar varnish is also sometimes apt to become cloudy after a time, and it is difficult to make.

TO MOUNT IN CANADA BALSAM OR DAMMAR VARNISH.

The sections having been properly stained and washed, are placed in methylated spirit to remove some of the water, and then immediately transferred to a small quantity of absolute alcohol in a watch-glass, and covered with another to prevent evaporation. They should be left in this for about 10 minutes. The absolute alcohol, which should be the strongest, sp. gr. ·795, has a great affinity for water, and will remove all that is in the sections.

When ready remove the sections one by one from the absolute alcohol with a needle, and drain off as much alcohol as possible by touching the section on the back of the hand or on a piece of clean filter paper; the back of the hand is the best, as some fibres from the filter paper may adhere to the section, which when seen under the microscope, will not improve the beauty of the preparation; when sufficiently drained, without being allowed to become absolutely dry, they are placed in a vessel containing oil of cloves; they will spread out on the surface of the oil, and as the spirit evaporates they will become completely permeated with it and very

transparent. If there are any folds these should now be straightened out carefully with needles.

Having placed a drop of Canada balsam on the slide, spread it out slightly with a needle, select a section in the oil of cloves, and pass the copper lifter under it, raise the lifter and hold the section in position with a needle by its upper corner, and having made sure there are no folds, remove the lifter with the section on it from the oil of cloves, let as much oil drain off as possible against the side of the vessel, and remove the rest by placing the edge of the lifter on a piece of filter paper. Place the edge of the lifter on the slide in the drop of Canada balsam, and gently draw down the section with a needle, as soon as a corner projects from the lifter on to the slide, hold it there lightly with the needle and slowly draw away the lifter; if this is carefully done the section will lie in its place in the middle of the slide without any folds.

A lifter is made by beating out the end of a copper wire, filing it smooth, and then turning up the broad portion slightly.

Take up a cover glass with the broad pointed forceps and hold it between the thumb and fore-finger of the left hand, place a small drop of Canada balsam on its lower edge, transfer to the right hand and gently lower it on to the section, keeping the left thumb against one corner to prevent its slipping, and gradually lower it with the forefinger of the right hand very slowly, watching all the time to see that no air bubble is entangled in the section.

With a little practice this can be done very neatly without an air bubble in any part of the preparation; it requires patience, however, and it is no use to try air pumps or any dodges, to remove the bubbles, as they

are useless; the only thing to be done when an air bubble lodges in a cavity of the section and refuses to move in any way by gentle pressure is to lift the cover glass, and transfer the section to oil of cloves, and then remount it.

When several sections are to be mounted on one slide, a slight pressure on each with the needle will generally retain it in its position, if too much of the mounting fluid is not used.

It will often be found on examining preparations after they have been mounted some little time, that the fluid has evaporated and left a vacuum under the cover glass; in this case a drop of the mounting fluid must be placed on the slide in contact with the cover glass, and it will immediately run in and fill up the empty space, provided always an egress has been allowed to remain for the contained air; when this is impossible from the small size of the hole at the edge of the cover glass, the only thing to be done is to wait until some of the material, of which the mounting fluid is composed, has been dissolved by the fresh fluid. Applying heat will effect it, and at the same time in all probability ruin the specimen.

Each preparation should be examined under the microscope and if found to be worth keeping, labelled. On the label should be noted the tissue, date of its preparation, mode of hardening and staining, thickness of cover glass if it has been measured, and anything of note which may be seen at the time it is examined. Exceptionally good sections should always have a private mark to show that they are not to be given away or exchanged.

They should be kept in a cabinet where they may lie flat.

ON BREAKING DOWN OLD PREPARATIONS.

It is often necessary to break down an old preparation and remount it. The cover glass may be broken, the staining faded, or the cover glass may be too thick, and preparations should never be discarded for these reasons, as it is quite easy to remount them. When a specimen has been mounted in glycerine, it is an easy matter to remove the cover glass, all that is necessary being to cut round the cement with a sharp knife, lift the cover glass carefully with a needle, and float off the section in water; if it is very delicate the cover glass had better be removed under water. The section can then be washed, to remove the glycerine, and re-stained if required; it will then be ready for mounting in the usual manner.

To break down a specimen mounted in Canada balsam or Dammar varnish is more difficult, especially if it has been mounted long enough to allow the balsam or Dammar to become hard. It must be placed in a bath of chloroform until it becomes soft enough to remove the cover glass, and this may be facilitated by passing the slide over the flame of a spirit lamp so as to heat it very slightly, but this requires care as the section may be easily ruined.

After the cover glass has been removed the section must be floated off into chloroform until all the balsam or Dammar has been dissolved out of it, and then placed in alcohol for a short time; it may then be re-stained if necessary and mounted again.

MOUNTING LARGE SECTIONS.

In manipulating large sections it is rather difficult to pass them through the different processes without injury. This may generally be done with care, and they may even be double stained.

There are some tissues, however, so fragile that they cannot be lifted on the needle without tearing, and these must be left in one vessel and the different processes applied to them there. This is not a very satis factory method, as the staining cannot be so well done unless a large quantity of fluid is used, and every section carefully separated from its neighbour, they are also apt to be injured in pouring off the different fluids. This is only required in exceptional cases.

It is when the mounting from the oil of cloves comes that the difficulty is experienced; as, even if a lifter is specially made large enough to take up a whole section, the adhesion of the section to such a large surface is so great, that it is impossible to get it off without tearing, if the section is as thin as it ought to be. It may, however, be done by using the cover glass as a lifter in the following manner.

Take as an example a longitudinal section of kidney of large dog or man; having been safely stained, it lies in the oil of cloves ready to be transferred to the slide.

The section measures say about $1\frac{3}{4}$ inches by 1 inch, some slides must be procured 3 by 2 inches and some cover glasses 2 by $1\frac{1}{2}$ inches, these had better be of No. 3 thin glass.

Having cleaned one of the slides, place some Canada balsam on it and spread it out with the needle, to something near the size of the specimen, then take the cover

glass and pass it into the oil of cloves under the specimen, in the same way the copper lifter is used to smaller sections; lift the cover glass and keep the section in its place, then drain off the superfluous oil by holding the cover glass on filter paper; on lifting it first from the oil it should be allowed to drain slowly from one corner, then invert the cover glass with the section on it, place a little Canada balsam at the lower edge, and lower it gently into the Canada balsam on the slide; this must be done very carefully, as bubbles will be found here and there, and the cover must be lifted a little and lowered again, until they have all been driven out. It is a tedious process, but amply repays the trouble.

The great drawback in this method is that the front of the cover glass is covered with oil of cloves and cannot be cleaned until the balsam sets, a matter of time with such a large surface. It can certainly be sealed up with Hollis's glue, but even then it is not safe, and requires a great many coats before the glue is sufficiently strong to resist such pressure as is required to clean the cover glass.

With some tissues it is possible to use a large cover glass as a lifter, and by allowing a large quantity of oil of cloves to remain to draw it off on to the slide and cover in the usual way, but with other tissues, such as Testis, this is utterly impossible if the sections are thin, and they can only be mounted in the manner first mentioned.

THIN SLIDES.

For preparations to be examined, under very high powers, thin slides made from glass called 9 or 10 oz. crown are useful, as they allow the condenser to come close to the object.

CHAPTER VII.

ON INJECTING THE VASCULAR SYSTEM.

THE materials used for this purpose are Berlin blue and carmine. Berlin blue is used either in suspension in water or in warm gelatine. It is made in the following manner, after the formula of Brucke :—

Solution A. Dissolve 217 grammes of ferrocyanide of potassium in a litre of water in a large flask.

Solution B. In another flask make a solution of chloride of iron containing one part of the salt in ten parts of water.

Solution C. Make a saturated solution of sulphate of soda.

Take 1 litre of solution A and mix it with 2 litres of solution C.

Take 1 litre of solution B and mix it with 2 litres of solution C.

Pour the B + C mixture slowly into the A + C mixture and stir constantly while doing so.

Allow the precipitate to settle and pour off the greenish supernatant fluid.

Pour the residue into a coarse filter or flannel bag. The blue liquid which comes through is returned to the strainer until it becomes clear.

Then wash what remains on the filter with water

thoroughly until what passes through is of an intense blue colour.

The filter is allowed to drain completely and then placed between sheets of coarse blotting-paper in a cool place, and left to dry gradually. The drying process may be facilitated by changing the blotting-paper as it becomes damp.

When thoroughly dry the material is broken up and kept in a stoppered bottle. A 2 per cent. solution of this material can be injected with great facility.

When it is used with gelatine, take 5 parts of the 2 per cent. solution in water and filter. Add this to 100 parts of a solution of gelatine, containing 1 part of gelatine to 8 parts of water.

To make the gelatine solution, dissolve it in a porcelain evaporating dish over a water-bath; when dissolved filter through muslin and replace in the water-bath. Add the blue solution gradually, with constant stirring (Klein).*

TO MAKE THE CARMINE GELATINE.

Suspend 4 grammes of carmine in a little distilled water, then add 8 c.c. of liq: ammonia and 48 c.c. of water.

Filter. Make a solution of gelatine, 1 part in 8, and filter through muslin.

Take 2 ounces of this and place it in a porcelain dish over a water-bath. Stir constantly, and add slowly the carmine solution. Add 50 minims of glacial acetic acid to $\frac{1}{2}$ an ounce of the warm gelatine solution, and mix gradually with the rest, stirring it all the time.

* See *Handbook of the Physiological Laboratory.*

Notice the solution carefully and the colour will be seen to change to a dirty red just before the last drops are added. This shows the slight acid reaction which prevents the carmine from being diffused through the walls of the capillaries (Klein).*

The apparatus required consists of a bottle with an opening at the top and another at the side, near the bottom—a two-necked Woolf's bottle; each of these should hold half a gallon. A small Woolf's bottle with three necks, the middle one stoppered; some glass and india-rubber tubing of different sizes.

Three screw clips; some ligature silk; an aneurism needle.

Two sharp-pointed forceps for dissecting; a water-bath for keeping the injection mass at the right temperature.

Sponges, scalpels, scissors, glass canulæ of different sizes.†

INJECTION APPARATUS.

Fix a metal eye into the ceiling of the laboratory; brass eyes with a long shank made for chandeliers are the best, as they can be screwed into a joist. Into this eye hook a small block; in this reeve a line with a running eye spliced in one end. A cleet will be required in the wall to make the line fast to when the pressure bottle is pulled up into position. Take the bottle with an opening at the top and bottom, and pass the running eye round the neck of the upper opening. Into the lower fit an india-rubber cork, with a piece of stout glass tube

* See *Handbook of the Physiological Laboratory*.

† The bottles, &c., can be procured from Griffin, Garrick Street, Covent Garden, or Orme & Co., Barbican. The canulæ from Stevens & Sons, Gower Street.

bent at right angles in it. Over this tube slip one end of a piece of india-rubber tubing long enough to reach the table, and fasten it tightly to the glass tube with a piece of string. Slip a screw clamp on to the tubing. Next take the two-necked Woolf's bottle of the same capacity as the first, and fit two india-rubber corks; into one pass a piece of glass tubing, bent at right angles, the long end of which must almost touch the bottom of the bottle. Into the other place a similar piece, but which only just reaches below the bottom of the cork. To the first-mentioned piece of glass tubing connect the long india-rubber tube from the pressure bottle, and to the other a short piece; fasten them both securely. This short piece must then be connected with a Manometer.* It is done in this manner. A short piece of tubing is put on the end of the glass tube of the manometer, the other end being attached to a glass T piece. The short tube from the large two-necked Woolf's bottle is then fastened to one end of the T, and another tube to the other. This last is fastened to one of the glass tubes in a smaller Woolf's bottle containing the injection mass.

The small Woolf's bottle for the injection mass should hold from 8–16 ozs., according to the size of the animal to be injected. It should have three necks, the centre having a glass stopper, the other two having india-rubber corks, in one of which a piece of glass tube, bent at right angles, is placed, so that it reaches nearly to the bottom of the bottle; in the other a short piece, reaching just below the cork. To this short piece the tubing from the T piece of the manometer is connected. A piece of fine india-rubber tubing is slipped on the other.

This should not be too large, as it has to go on the small glass canula in the blood-vessel.

* Made by Hicks, Hatton Garden.

A screw clip is slipped on the tubing, between the manometer and the Woolf's bottle, containing the injection mass, and another near the end of the tubing that is to go on the canula.

Glass canulæ of different sizes are required; they are difficult to make, but the process is described and figured in the "Handbook of the Physiological Laboratory;" they can be bought of any size from Messrs. Stevens & Sons, surgical instrument makers, Gower Street. As soon as the apparatus has been put together, all india-rubber tubing should be securely tied on the glass tubes. The apparatus should then be tested to see if there is any leakage. To do this, fill the pressure bottle, and pull it up about eight feet from the ground, screw up the clip between the Manometer and the small Woolf's bottle; then open the clip on the pressure bottle, and let the water run into the large Woolf's bottle until the Manometer registers three inches. Let it stand for five minutes, and see if there is any fall in the mercury. If not, screw up the clip close to the canula, and open the one between the small Woolf's bottle and the Manometer. If there is no fall of the mercury after this has been done some little time, the apparatus is in good working order. If the mercury falls, some of the corks are probably not fitting tightly, or the india-rubber tubes are not fastened properly.

To Inject a whole Animal.—Kill with chloroform; cut out a small portion of the left side of the thorax over the heart, taking care not to injure the internal mammary artery. Take two pair of sharp-pointed forceps to do the rest of the dissection. Pick up the pericardium, cut it open, and pass a piece of silk ligature through the apex of the heart; open the left ventricle and sponge away all the blood, dissect out with the forceps the root of the

aorta, and pass a ligature round it with an aneurism needle. Pass a glass canula through the opening in the wall of the heart into the aorta and tie the ligature round the constriction in the neck. Open the right side of the heart.

An assistant should hold the piece of silk ligature passed through the apex of the heart, so that it may be pulled down a little and kept steady.

The small Woolf's bottle, having been filled with injection mass, and kept warm in a water bath, is now connected with the pressure apparatus, and the stopper firmly fixed in the middle neck. The clip next the canula is now screwed up, and the one behind it opened, so that the pressure is brought to bear on the injection mass. The animal having been placed in position, either raised on a board, or as most convenient, the canula in the aorta is cleansed from blood, and filled up with some warm $\frac{1}{2}$ per cent. salt solution. The clip is then opened a little to let the tube fill with injection mass to the end, and the tube is slipped on to the canula and fastened; the clip is then fully opened. The pressure registered by the Manometer is carefully watched; it must be low to begin with, about one inch on each arm, and this must be increased gradually, as the injection progresses, by letting more water run from the suspended pressure bottle until about two inches are registered. At the very end, a little more than that may be given, but the amount can be best found out by practice.

On examining different parts of the animal it will easily be seen how the injection is progressing.

As soon as the injection mass begins to come out of the right side of the heart, a ligature should be tied round the whole base of the heart.

When the whole animal appears to be of a uniformly

red hue in those parts that will show it the pressure may be increased for a short time, and then a ligature passed round the tubing and tied tightly.

The animal is then removed to a vessel containing spirit, in which are some blocks of ice.

It must remain in this some hours, until the injection mass has become solidified throughout.

The animal can then be dissected, and the parts hardened in the usual manner.

To Inject a Single Organ.—Take, for example, the liver of a rabbit. The thorax and abdomen are carefully opened up, and portions of the wall of the thorax removed, until the superior vena cava is thoroughly exposed; a ligature is passed round it, and lightly tied. The stomach is then lifted up, and the portal vein will readily be seen beneath it; a ligature is passed round it, and a slit made with a pair of fine scissors, a canula is inserted and tied in. The tubing from the bottle containing the injection mass is now slipped on, and securely tied, and pressure applied. A slit is now made in the superior cava to let out the blood. As soon as the organ has become distended and firm, tie the superior cava, and after a little time the tubing outside the canula. Cut the tubing, and remove the whole organ very carefully, and place it in cold spirit. As soon as the injection mass has completely hardened, the organ may be cut up and placed in chromic-acid mixture, and hardened as usual.

To Inject the Bile Ducts, as well asthe Blood-vessels.—To do this it is necessary to have two Woolf's bottles fitted in the manner already described, but to connect them with the pressure apparatus a T piece must be placed on the tubing coming from the Manometer, and the two smaller bottles connected the same as before,

only each must have a screw clip between it and the Manometer, to shut off the pressure from the one while the other is in use.

Having the apparatus all ready and the two small Woolf's bottles filled with injection mass, the one carmine gelatine, the other Berlin blue and gelatine, shut off the red by the screw clip. Then carefully dissect out the common duct, and insert a canula through a small slit and tie it in. This canula must be very small; fill up the canula with warm Berlin blue 2 per cent. solution, and slip on the tubing, tie and put on the pressure. The pressure must be increased gradually to a higher degree than in the case of the blood-vessels, as the injection is going where there is no outlet, and the bile in the ducts has to be pushed as far as possible to make the process a success. When no more injection mass will go in, and the organ appears blue in patches, tie the tubing and cut it. Screw up the clip on the blue, then dissect out the portal vein, insert a canula and tie it in, and proceed as before directed for a single organ. When the liver appears to be fully distended with the injection, tie the tube and cut it. Then remove the whole organ carefully and place it in cold spirit for a time, until the injection mass has completely hardened.

In the winter some difficulty will be found in keeping the injection mass from solidifying, especially in the tube. To obviate this, the bottle must be kept in a water bath on a gas stove, and sponges dipped in hot water should be laid on the tube and on the organ to be injected. With a little thought this can easily be done and the injection made without any complicated apparatus for keeping the whole thing warm.

TO INJECT THE LYMPHATICS WITH A COLD SOLUTION.

It is often required to demonstrate the lymphatics in some organ. To do this a cold 2 per cent. solution of Berlin blue is used. A Pravaz syringe is filled with the solution and the piston pushed down, so that a drop is forced out at the point, showing that there is no air in the canula. Thrust the point a short distance into the tissues and push down the piston gradually. If the point is in a lymph channel, the blue will soon make its appearance in fine lines. If not withdraw a little and shift the direction. The piston must be pushed down very slowly. In this manner the lymphatics become filled with the blue, and the organ can be hardened in the usual manner.

CHAPTER VIII.

METHOD OF OBTAINING ANIMAL TISSUES FOR EXAMINATION.

THE animals required will be a cat, rabbit, and guinea-pig; and for some special tissues, a frog, salamander, and newt.

To kill the first three animals, place them in a box with a tight-fitting lid, having previously introduced a sponge saturated with chloroform. In this confined space the chloroform will render the animals insensible in a very short time, and then kill them without inflicting the slightest pain.

The other three animals may be chloroformed under a small bell glass on a plate.

Having killed either of the first animals, make an incision through the skin, from the chin to the anus, and reflect it on each side, open the abdomen and remove some of the mesentery and omentum for preparation by the silver process; open the thorax and make an incision into the heart and drain off the blood before it coagulates. Before opening the heart a small piece of the centrum tendineum of the diaphragm may be carefully cut out to be prepared by the silver process. Then open up the thorax and abdomen thoroughly. Take out the lungs with a small portion of the trachea

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attached. Remove the heart, open up the ventricles. Remove the liver and cut it into small pieces. Take out the stomach with a piece of œsophagus and duodenum attached, open it longitudinally and wash it in dilute solution of chromic acid to remove particles of food, &c.; this must be done gently, and it must not be rubbed. Open the intestines with scissors, and wash in dilute chromic acid; cut into short lengths. Cut out carefully the ilio-cœcal valve with a portion of intestine on either side. Remove the kidneys, and open one longitudinally, the other transversely, in two or three places. Take out the spleen and pancreas and cut them in small pieces. Take out some of the mesenteric glands; remove carefully the uterus and ovaries if a female. Open the scrotum and remove the testes, in many cases these can be got out more easily by pushing them up into the abdomen. Dissect out the penis and remove it. Then cut round the skin of the neck and disarticulate the skull, taking care to leave the remaining portions of the trachea and œsophagus. Disarticulate the lower jaw and remove the tongue with the trachea and œsophagus; cut off these at the base of the tongue, then divide that organ longitudinally, leaving one side whole, make transverse cuts in the other. If large it will be necessary to make some longitudinal incisions in the first for hardening with chromic acid; but this should be done so as not to interfere with making longitudinal sections of the whole of one side. Take out the submaxillary glands. The eyes and brain will then only remain, and great care must be exercised in taking them out.

The best plan is to remove the brain first; for this purpose take off the covering of the nasal organ with a pair of bone forceps, and snip off pieces of bone from

the upper part of the skull, taking care not to touch the brain underneath, at the same time care must be used not to squeeze the eyes in holding the skull. When all the upper part of the brain is laid bare, the bone should be nipped through in front of it with the forceps, and the base of the skull divided; by gently cutting away the bone by degrees and dividing the nerves proceeding from the brain, it can be completely freed with the cerebellum, pons, and medulla intact.

It requires some practice and care to do this well, but even if the brain is a little torn in removing it, there will still be plenty to harden and make sections of. The bone can now be cut through, round, and in the orbit the optic nerve and recti muscles divided, and the eye removed whole.

Portions of nerve can be taken from different parts, the optic, sciatic, &c. Portions of muscle may also be cut out. The aorta and some of the larger arteries should be removed and cleaned from the surrounding tissues.

DISSECTION OF FROG.

The whole eye may be removed from a frog and placed in Muller's fluid, but the cornea is required to make a gold preparation.

Having killed a frog, wrap it in a cloth and render the eye tense with the thumb of the left hand. Insert one of the points of a fine pair of curved scissors at the edge of the cornea and cut it round carefully, separate it from the rest of the eye, and place it in gold chloride.

The mesentery and meso-gastrium may be prepared by the silver process.

DISSECTION OF NEWT AND SALAMANDER.

Kill a newt and cut off the head, fix it to the table on its back, by a needle passing through the neck and another through the tail. Make a longitudinal incision down to the anus, take up the upper end of the stomach with a pair of forceps, and by carefully cutting away the connective tissue attaching it to the cavity, the whole of the contents of the body can be removed *en masse.*

Cut away the lungs and liver, which are not wanted. Make an incision through one side of the stomach in its whole length, and then put the whole in solution of chromate of ammonia. In Triton cristatus there will generally be found in the male, two testes on each side, but sometimes three or four; some of these may be removed for the purpose of making a fresh preparation of spermatozoa.

Salamander is treated in the same way for the same tissues.

PRACTICAL HISTOLOGY.

BLOOD.

TAKE a small drop of newt's blood, cover and examine: notice the difference in shape and number between the coloured and white corpuscles. Prick the finger and examine human blood in the same way.

AMŒBOID MOVEMENT.

A warm stage is required to show the amœboid movement of the white blood corpuscles.

With a camel's hair pencil apply a little oil round the edge of a cover glass, place a small drop of perfectly fresh newt's blood in the centre of the glass, and cover with another; lay the preparation on the warm stage over the central hole, and apply the spirit lamp to the wire. The thermometer should rise to 30° C.

Select one of the large colourless corpuscles, and sketch the different movements shown by it at distinct intervals. Make a similar preparation of human blood, and examine in the same manner. The cover glass must be warmed for human blood, and the top cover glass should be touched on the spot of blood coming from the pricked finger. The coloured blood corpuscles will form rouleaux if properly prepared.

A preparation of fresh newt's blood should be examined with a high power on the warm stage to see the

beautiful intra-nuclear and intra-cellular network of fine fibres in the red corpuscles.

FEEDING BLOOD CORPUSCLES.

Rub up vermilion cake in $\frac{3}{4}$ per cent. salt solution, add a very small drop of this to the blood on a slide, cover and examine on the warm stage, paint a little oil round the edges to prevent evaporation.

In the case of newt's blood the thermometer should not rise above 30° C., and in that of human, not above 40° C. After a time the white blood corpuscles will be found to have enclosed some of the vermilion granules. Newt's blood from the larger size of the corpuscles will show this best.

IRRIGATING BLOOD CORPUSCLES.

Make a $\frac{3}{4}$ per cent. saline solution of newt's blood on a slide, cover and examine: notice the red corpuscles gradually becoming crenated. Try the effect of irrigation on this preparation, place a small piece of filter paper in contact with the edge of the cover glass, and with a capillary tube place a small quantity of the fluid to be introduced on the opposite side; as the filter paper withdraws fluid from one side, the new fluid will flow in from the other. When thicker fluids, such as glycerine are to be removed, a capillary tube is necessary on one side to remove it by suction with the mouth.

The fluids to be tried by irrigation are:—Dilute acetic acid; distilled water; 2 per cent. solution Tannic acid; 2 per cent. solution Boracic acid. For their effect the student is referred to the "Atlas of Histology" by Dr. Klein.

On staining blood corpuscles. Blood corpuscles may be stained and mounted as permanent preparations.

A little blood is dried on a slide, and when thoroughly dry exposed to the fume of a strong solution of osmic acid, or a little of a 1 per cent. solution is placed on the blood and allowed to remain for a minute or two; many other agents will do, as the only thing requisite is to coagulate the albumen, otherwise the blood corpuscles will lose their natural contour. It is necessary to be careful if some of the salts are used, as they may leave crystals amongst the blood corpuscles which stain, and are apt to be mistaken for Bacteria. Wash off the osmic acid solution with distilled water and drain, then cover the blood with a little of the staining solution and allow it to remain for a few minutes, the time must be found out by practice, as it will vary with the stain used and the thickness of the film it has to penetrate.

Gentian Violet and Rosanilin Acetate are the best staining agents. When sufficiently stained wash in water, and then in methylated spirit until no more colour comes away, allow it to dry and then mount with Canada balsam. The blood may be washed thoroughly in water, and then mounted in a solution of acetate of potash just under saturation, it must then be sealed with Hollis's glue.

HÆMIN CRYSTALS.

These are interesting as demonstrating the presence of blood, and they are very easily prepared, and can be sealed up and kept as a permanent preparation. Glacial acetic acid decomposes the blood pigment, and forms a hydrochlorate of hæmatin in the presence of sodium chloride.

Place some ordinary table salt in a watch glass, and

hold it over a spirit lamp until it is thoroughly dried. Take equal parts of any dried blood and this dried salt on a slide, put on a cover glass, and with a capillary tube run in glacial acetic acid, warm it gently over a spirit-lamp until a good deal of the acid has evaporated, and then examine under the microscope. To mount this as a permanent preparation, wash out with distilled water all the glacial acetic acid, and seal with Hollis's glue. It is better to use some rather largish masses of blood, or when the acid is being washed out all the crystals will go as well, the larger masses will be held by the weight of the cover glass, and their margins will be found covered with Hæmin crystals.

EPITHELIUM.

SQUAMOUS EPITHELIUM.

With a blunt knife scrape a little saliva from the back of the tongue or inside of the cheek, cover and examine. Look for squamous epithelium, the so-called salivary corpuscles showing Brownian movements of the granules they contain, micrococci and in many cases bacteria.

Shed skin of newt. This makes the best preparation of surface epithelium. Place a newt in a glass jar of water, and in three or four days it will be found that it has shed the entire outer layer of epithelium as a continuous skin; unroll it carefully, and cut into small pieces, stain with logwood and mount in glycerine. The staining will be facilitated by first placing it for a short time in slightly acidulated water. Seal with Hollis's glue, and examine with a low power. The

outlines of the cells and their nuclei will be very well shown.

Make a vertical section of skin prepared in chromic acid mixture (wherever this mixture is mentioned, solution No. 1, p. 15 is meant). Stain in logwood and mount in Canada balsam.

Observe the different layers of cells, and with a high power seek for the prickle cells of the Rete Malpighi. These are, however, better seen in some epithelial Cancers.

COLUMNAR EPITHELIUM.

Take the stomach of a newt or salamander prepared in solution of chromate of ammonia (page 17), and wash well until no colour comes away, place the whole in a dilute solute of picro-carmine, about 15 drops to a watch glass of distilled water, and let it remain until it has taken on a deep red colour, then remove and wash off the excess of colouring matter; scrape off a little of the surface material from the inside of the stomach, and tease out gently on a slide in a drop of glycerine, cover and examine.

With a high power the network in the cells and their nuclei will be seen as depicted in the Atlas.

Make a longitudinal section of the large intestine of cat, dog or rabbit, prepared in chromic acid mixture, stain with logwood, mount in Canada balsam, and examine. The columnar cells will be seen in rows, some of them having become goblet shaped—that is, distended by their secretion, and if the animal has been killed some time after feeding, when digestion is going on, these goblet cells with stain deeply with logwood, and the mucin will be seen poured out from the cell in deeply-stained masses.

If, however, the animal has been killed within a short time of feeding—that is, before the mucigen has been changed into mucin, these goblet cells will not stain with logwood, but will stain with anilin colours.

CILIATED COLUMNAR EPITHELIUM.

Take a portion of the trachea of cat, prepared in 2 per cent. solution of bichromate of potash, wash well in water, and stain the mass in logwood; it must be put in a strong solution and left for several hours; when deeply stained remove it, wash well until no more colouring matter comes away. Then with a small knife scrape away a little of the inner surface, and place it in a very small drop of glycerine on a slide, pound it with the rounded end of a needle-holder until the whole drop is seen to be permeated with fine particles, and no large ones are left; place the cover glass on gently and allow the fluid to spread under it; seal and examine.

The ciliated cells will be found isolated and can be readily examined. Two other varieties of cells will be found, which are the cells of the deeper layers and they are not ciliated; try with the $\frac{1}{6}$ to make out the striation of the line running across the cell at the base of the cilia; a good glass will show this; the $\frac{1}{12}$ oil immersion will show that this striation is caused by the cilia, which are continuous with the longitudinal striation in the body of the cell.

Make a transverse section of the epididymis of man or dog prepared in chromic acid mixture and stained with logwood. Here the ciliated cells will be beautifully shown, the cilia being much longer than in the trachea; they can be traced through the striated border,

with a high power, in the same manner as in the cells of the trachea.

ENDOTHELIUM.

Take a portion of mesentery of cat and prepare by the silver process. When it has been left long enough in distilled water, stain it with logwood and mount in glycerine; seal and examine.

The nuclei will be well shown by the logwood stain, but the outlines of the cells will not probably be very distinct at first, but will become more so by the action of light. It will then be seen that the silver has been deposited in the intercellular substance between each cell, giving it a dark border. By focussing carefully a second layer of cells will be brought into view, whose outlines do not correspond with the first; these are the endothelial cells on the other surface of the membrane. In some places masses of small cells will be found deeply stained, these are germinating cells.

Other serous membranes should be prepared by the silver process, the centrum tendineum of the diaphragm makes a very good preparation, showing the groups of germinating cells, and the difference in shape of the endothelium on each surface.

The lymphatic capillaries will also be seen by the different shape of the endothelium, giving them the appearence of trabeculæ running through the membrane.

A silvered preparation of the septum cisternæ lymphaticæ magnæ should be made.

Remove the viscera from a recently killed frog, and a large lymph sac will be found on each side of the spinal column behind the stomach, the septum separates each sac from the peritoneal cavity. It may be stained *in*

situ by pouring a little ½ per cent. solution of nitrate of silver over it and allowing it to remain a few minutes, or the septum may be carefully removed and placed in silver solution. The septum will show the germinating cells round the openings of the stomata. In all cases where preparations of serous membrane are to be made, the animal should be bled first.

CONNECTIVE TISSUE CORPUSCLES.

Make a gold preparation of Tadpole's tail.

This will show the connective tissue corpuscles very well with their branched processes. Pigment cells are also numerous. Several of these preparations should be made, as they show a great many different structures.

Make a preparation of newt's mesentery in 5 per cent. chromate of ammonia (page 17), and double stain with picro-carmine and logwood (page 38). In this preparation very larged branched corpuscles will be seen having the hyalin ground plate stained with logwood; these make most beautiful objects for examination with high powers.

The corneal corpuscles will be mentioned in another place.

TENDON.

To show the tendon cells which lie in the interfascicular lymph spaces, take a young mouse just killed and remove the skin of the tail, then with the fore-finger nail separate two of the caudal vertebræ and forcibly remove the distal portion. Several white threads will be left, these are the tendons. Take a small bit of one of the finest and place it in slightly acidulated water

for a short time, then remove to half per cent. gold solution, let it remain about twenty minutes. Then place it in distilled water, which must be changed once or twice, until it becomes a brown colour. Take a small bit and place it in a drop of glycerine on a slide, and separate it into as many fibrils as possible. Cover and examine.

Take the tail of a young rat and prepare it in gold chloride (page 18). Make transverse sections and double or treble stain them. The tendon cells will be seen, darkly stained with the gold, lying between the bundles of fibrous tissue forming the tendons.

Tendon should also be examined in the fresh state, by taking a small portion from the mouse's tail and mounting in salt solution, then irrigating it with very dilute acetic acid, and watching the change that takes place, as the fibrous tissue swells up and becomes indistinct, the cells becoming granular.

The acetic acid should be only just sour to the taste.

After a time the whole of the fibrous tissue will have disappeared, leaving a very few elastic fibres which are untouched by the acid.

Take also some of the fresh tendon and place it in logwood stain, to which a few drops of glycerine have been added; let it remain until deeply stained. Tease out small portions in glycerine on a slide; the tendon cells will be well shown by this process.

ELASTIC TISSUE.

Make a preparation of mesentery of frog and mount in glycerine; a very fine network of elastic fibre will be found throughout the whole structure.

Take a small slice of the ligamentum nuchæ of the

ox, which can be readily procured from the butcher's. Place it in dilute acetic acid for some little time, until it swells up, then tease a small portion in a drop of glycerine on a slide. Cover and examine.

WHITE FIBROUS TISSUE

Is well shown in many of the preparations of serous membranes. A special preparation should, however, be made, by hardening omentum in 1 or 2 per cent. bichromate of potash and staining with logwood ; it will show the large amount of fibrous tissue present in a serous membrane, forming the greater part of the framework.

White fibrous tissue is also well seen in sections of skin ; also in submucous tissues.

ADIPOSE TISSUE.

Well seen in some of the preparations of serous membrane, when the fat cells lie thickly along the sides of the blood vessels. Also seen in cutis vera, and in many other parts. It is not necessary to make a special preparation of it.

A serous membrane placed for a short time in dilute osmic acid, and mounted in glycerine, will show the fat cells differentiated from the surrounding tissue, as they have all become blackened by the action of the osmic acid.

CARTILAGE.

HYALINE CARTILAGE.

The thin cartilaginous expansions from the sternum of the newt, prepared by the gold process, make very good specimens of hyaline cartilage. Thin sections may be cut by the microtome, or by hand. In this preparation the lymph canals will be seen looking like dark processes proceeding from the lacuna, in which the cell lies, into the hyaline matrix.

Sections of the nasal cartilages of small animals, growing bone, &c., will all give good examples of hyaline cartilage.

In the fresh state the cells will be seen to fill the lacunæ; but in hardened specimens they have all shrunk, more or less, leaving a space.

Specimens of cartilage should also be hardened in chromic acid mixture, and thin sections stained with logwood.

FIBRO-CARTILAGE.

Make a longitudinal section of mouse's tail, and notice the intervertebral cartilage and its gradual transition into hyaline cartilage on the bone. The fibres will be seen in various aspects as they cross one another, and several sections should be examined; the cells will be seen lying between the fibres.

Make a section of the intervertebral disc of sheep or ox, hardened in chromic acid mixture, and stain with logwood. Mount some sections whole and tease out others on the slide. Cover and examine. It will be difficult to make out the fibres in some sections.

ELASTIC CARTILAGE.

This can be well shown in the lobe of the ear or in the epiglottis. Procure the epiglottis of a sheep and harden it in the chromic acid mixture, cut sections and stain them with logwood.

The ear of a child prepared in the same manner, sections cut and stained with logwood.

The ear lobe of a pig also prepared in chromic acid mixture and stained with logwood. Sections of these must be thin and they must not be hardened too much or it will be difficult to cut them.

The pig's ear when well prepared makes a very useful specimen, as it shows a great many tissues.

BONE.

PREPARING HARD BONE.

Bone must be examined in two forms, first in its dry state, and secondly when it has been decalcified or had its earthy salts removed. In the case of dry bone a very few sections will suffice as it is a difficult and laborious task to get them well made.

The bone is fixed in a vice and sections as thin as possible are cut with a fine saw, these are rubbed down with fine emery on a stone, and finally polished on a hone; they must be well washed to remove all debris and are better mounted dry, as they are apt to become too transparent when mounted in glycerine or other media.

DECALCIFYING BONE.

It is a very different matter to make sections of a bone after the earthy salts have been removed, as it can be cut, as easily as any other tissue, with the freezing microtome or razor.

To macerate small bones, such as mouse tail, half per cent. chromic acid will be sufficient; they should not be left in too long. Larger bones must be cut into small pieces and placed in half per cent. solution of chromic acid for a week or ten days, and then one-twentieth of the volume of hydrochloric acid added to the original fluid for large bones, less for smaller. First harden then soften them, five or six days after the addition of the acid will be enough according to the size of the bones. They should then be thoroughly washed in water for several days or a week, according to size, to get rid of the lime salts, and preserved in spirit.

Bone may be macerated in a saturated solution of picric acid, but it does not act so well as the above, it must be kept saturated by the addition of fresh crystals.

Make longitudal sections through the head of a long bone such as the femur of a small kitten, double stain with picro-carmine and logwood and mount in Canada balsam. Take a portion of the lower jaw of a very young kitten near the condyle and decalcify it. When prepared cut transverse sections and double stain them with picro-carmine and logwood and mount in Canada balsam. Sections also through the carpus and tarsus of a fœtal child or kitten may be made and stained in the same manner.

The easiest specimens of growing bone to be got are from kittens just born, they should be decalcified in chromic acid as mentioned before.

MUSCULAR TISSUE.

NON-STRIPED, STRIPED, HEART MUSCLE.

1. *Non-striped Muscle.* Make a preparation of the mesentery of newt or salamander in chromate of ammonia (page 17), and mount in glycerine. This specimen shows better when doubled stained with picro-carmine and log-wood (page 38).

Examine first with a low power to see the distribution of the muscle fibres through the mesentery, and then with a high power to see the structure of the individual fibres; with the one-sixth the network in the nucleus can be seen, but it will require a one-eighth or Zeiss' E to make out the fibrils passing out of the ends of the nucleus into the body of the cell. Look carefully also for the transverse markings on the cell and the difference of its diameter, in some places, compare their appearance with the plate in the Atlas of Histology.

These fibres are very large, and give a good idea of the structure of a non-striped muscle fibre.

In the large area in which this tissue is distributed throughout the body of a mammalian animal, the individual cells are very much smaller, and their structure cannot be made out without a high power, especially as they lie very thickly together. In the intestine, however, a thin section will show a few fibres running up from the muscularis mucosæ to the basement membrane, and in these when well prepared and stained the same structures can be seen as in the much larger ones in the mesentery of newt.

To see the intercellular substance by which the muscle

fibres are held together, take a small portion cut longitudinally from the intestine and prepare it in chromate of ammonia, page 17, and stain in logwood. Cut longitudinal sections so that the circular muscle coat of the intestine is cut transversely, and it will be seen that each fibre is separated from the others by a homogeneous substance—the intercellular cement. There will be only a few nuclei cut through, and these will be deeply stained.

2. *Striped Muscle.* Striped muscle is best shown in one of the large water beetles, Hydrophylus piceus. There is another water beetle often sold for this purpose which does not answer so well, the Dytiscus marginalis; it may be distinguished by being smaller and having a yellow line round the margin of the upper surface.

Fresh Preparation of Muscle to show the Sarcolemma. Kill the beetle and remove one of the legs, open the chitinous covering and snip off a bit of the muscle, place it on a slide in a drop of distilled water, tease it out, cover and examine. The sarcolemma will be seen in places raised from the muscle substance.

Irrigate the same preparation with dilute acetic acid and the nuclei of the muscle corpuscles will soon come into view.

To make a permanent preparation stain a small portion of the leg muscle with logwood, tease it out carefully into as fine fibrils as possible in a drop of glycerine on a slide, cover and seal up with Hollis's glue. Dissect out some of the muscles of the thorax and mount in the same way. Examine the muscles in a transverse section of the tongue of any animal prepared in chromic acid mixture and doubled stained with picro-carmine and logwood.

3. *Heart Muscle.* Sections made from the heart of any small mammal will show the peculiarities of this variety of muscle fibre. Make transverse and longitudinal sections, and stain them with logwood. Note the position of the muscle corpuscles in the transverse sections, and the anastomosing of the fibres in those cut longitudinally.

NERVOUS STRUCTURES.

MEDULLATED NERVE FIBRES.

Dissect out the sciatic nerve of a frog and stain for a few minutes in half per cent. solution of nitrate of silver, wash well and expose to the light in distilled water, until it has become of a brown colour; cut small portions and gently tease out so as to separate the fibrils, this must be done carefully in a drop of glycerine on a slide; cover and examine.

NODES OF RANVIER.

These will be found marked by black crosses in the course of the nerve fibre, the silver being heavily deposited at the node and penetrating a short distance either way, giving the appearance of a cross. These nodes can be seen in the fresh nerve, or in nerve hardened and stained in the ordinary manner.

HARDENING NERVES.

Take any of the larger nerves from the animals used for material and harden some in 2 per cent. bichromate of potash (page 17); others in chromic acid mixture.

Cut longitudinal and transverse sections of each of these and stain with logwood, mount in Canada balsam.

NON-MEDULLATED NERVE FIBRE.

This is best seen in a gold preparation of the tadpole's tail; it will require a high power to trace the finest fibres, a $\frac{1}{8}$ dry, but the one-twelfth oil immersion of Powell and Lealand with the binocular will enable the student to form a more correct idea of the structures he is looking at and their relation to one another.

SPINAL CORD.

Procure a fresh spinal cord of calf from the butcher and cut it into pieces corresponding to the different regions. Prepare one half of each of these pieces in a 2 per cent. solution of bichromate of potash, page 17, and the other half in chromic acid mixture; those prepared in bichromate of potash may be put in as they are, but those in chromic acid mixture must be cut into lengths of about half an inch. The cord must be carefully handled, care being taken not to squeeze it, and it should be cut with a sharp razor. Transverse sections of these must then be cut and stained with logwood, some may be stained with aniline blue (page 33), others double stained with picro-carmine and logwood (page 38).

Sections can be cut by the microtome, but the razor must be very sharp and the cord well hardened. Very thin sections can also be cut by the hand, and each section should be washed off the razor into spirit as it is cut. This should not be done with a brush but by dipping the razor into spirit. The cord prepared in

bichromate of potash will show the nerve structures, that prepared in chromic acid mixture the neuroglia.

TO SHOW THE LARGE MULTIPOLAR NERVE CELLS IN THE SPINAL CORD.

Cut a piece of hardened spinal cord through the middle longitudinally, take one side and holding it in the hand cut out with a sharp razor as thin a slice as possible of the anterior column, stain this deeply in picro-carmine. Take a small portion and place it in a drop of glycerine on a slide and tease out very carefully with two needles, this must be done gently and continued for some time, until the whole piece is reduced to very minute portions, hardly discernible except by their colour ; cover and seal up.

If this process has been carefully done, the large cells will be seen stained with picro-carmine and completely isolated with processes of different lengths, according to the care which has been exercised in teasing.

Several sections of the anterior column should be made, and after staining they should be placed on a slide and examined with a low power ; those which contain a large number of multipolar cells, will be readily seen.

BRAIN.

Small brains, such as rabbit's, harden very well whole by the spirit process (page 17), they must then be placed in absolute alcohol ; before doing this the different parts should be separated—viz. : frontal lobes, cerebellum, pons, &c.

After remaining in the alcohol a few days, sections may be cut of any of the parts to be examined and

stained with logwood, which shows the structural elements better than any other stain.

Human brain is best hardened in 2 per cent. solution of bichromate of potash (page 17); it must be well washed until no more colour comes away, before sections are cut. This method is equally applicable to all large brains.

Large sections of brain may be cut with the microtome, but for Histological work small sections will be found to show all that is required. It is a comparatively easy matter to cut a large section, but to pass this section through all the different re-agents and finally to get it laid out smoothly on the slide, without tearing, is a very difficult matter indeed.

Bichromate of ammonia (p. 17), may be used in the same manner as bichromate of potash, for all nervous structures.

To show the neuroglia of the brain, it must be cut in small pieces and hardened in the ordinary chromic acid mixture.

PACINIAN CORPUSCLES.

These are best seen in the meso-rectum of cat, where they are visible to the naked eye as oval bead-like bodies. Cut out a portion of the meso-rectum and spread it on a flat piece of cork, fasten it at the sides by a few pins, and invert the cork in a vessel containing a 2 per cent. solution of bichromate of potash; let it remain in this for a few days, then cut it into small pieces and wash well, place them in a very dilute solution of logwood, one drop to a watch-glass of distilled water will be enough. They must be allowed to stain very gradually, as the logwood takes some time to penetrate the capsules. The solution of logwood must be

changed several times, as it is apt to become granular. It will take about 48 hours, or longer, to stain the corpuscles thoroughly. When they have taken in the colour sufficiently, wash them well in plain water and mount in glycerine.

Vertical sections should also be made of the pad of a cat's foot, hardened in chromic acid mixture and stained in logwood.

BLOOD VESSELS.

CAPILLARIES.

Take the tail of a half-grown Tadpole of the common frog, and place it in a 5 per cent. solution of chromate of ammonia for 24 hours to remove the epithelium, then wash well until no colour comes away in the water, and double stain with picro-carmine and logwood (page 38). Mount in Canada balsam.

By this process the capillaries will be deeply stained with logwood, and can be seen in their natural condition.

Examine the capillaries, in a gold preparation, of a Tadpole's tail stained with logwood.

Either of these preparations will show them in process of development from branched connective corpuscles. Examine them carefully for the nuclei of the walls, and observe in many the contained blood corpuscles.

ARTERIES AND VEINS.

Take the aorta of the dog or cat and prepare either in chromic acid mixture or spirit mixture (page 15). Make longitudinal and transverse sections, stain some

with logwood and double stain others with picro-carmine and logwood (page 38). Mount in Canada balsam. Also make transverse sections of the whole aorta of rabbit, and of smaller arteries and veins of the same and other animals, prepared as above. Stain with logwood and mount in Canada balsam. Examine these carefully to see the varied amount of elastic tissue and non-striped muscle in the different arteries.

Veins are prepared in the same manner as arteries, but as they are so much slighter in structure, it is easier to examine them *in situ* in such sections as tongue, kidney, or skin.

ENDOTHELIUM OF BLOOD VESSELS.

To examine the endothelium of a blood vessel it should be opened and then placed for two or three minutes in half per cent. solution of nitrate of silver, and exposed to the light in distilled water until it has become a brown colour. Then pin it out on a cork and tear off with the fine pointed forceps thin strips of the intima. Mount these in glycerine.

To examine the different coats of a blood vessel separately, it must be macerated for a few days in a 2 per cent. solution of bichromate of potash, well washed, and strips torn off from the different coats; these can be then stained and mounted.

LYMPHATIC GLANDS.

The lymphatic glands of the cat are very good for examination, and should be perfectly fresh; they are best hardened in chromic acid mixture.

Thin sections should be made with the microtome

and stained with logwood. After washing, some of the thinnest should be placed with some water in a test tube, and shaken for half an hour or more, to detach the corpuscles from the adenoid reticulum. They must be shaken steadily or they will be knocked to pieces. Two or three sections only should be shaken at one time. They are afterwards mounted in the usual manner in Canada balsam.

It is a good plan to inject a solution of Berlin blue into the lymph channels of a lymphatic gland, to demonstrate the passage of lymph through it. It is done by inserting the point of a hypodermic syringe, filled with a solution of Berlin blue, through the capsule of a fresh gland, and slowly injecting the colouring matter; the gland is then prepared in the usual manner, and sections cut and stained with logwood.

THYROID GLAND.

This may be prepared in precisely the same manner as a lymphatic gland.

SALIVARY GLANDS—PANCREAS.

May be hardened in chromic acid mixture or in spirit mixture, and care must be taken that they are fresh and not over-hardened. Sections may be cut by the microtome or by hand and stained in logwood.

TEETH.

Sections of hard teeth are prepared in the same way as bone. Teeth may be decalcified by the same process as that used for bone (page 80). Good sections of teeth

in situ may be made by removing the lower jaw of some small animal, as rat or mole, and decalcifying it; after the lime salts have been thoroughly washed out, it should be soaked in gum for 24 hours and then cut with the freezing microtome.

Sections may either be mounted without staining in Canada balsam, or they may be double stained with picro-carmine and logwood.

ALIMENTARY CANAL.

STOMACH.

The stomach may be prepared in several different ways.

1st Method. Remove and open a fresh stomach of dog, cat, or rabbit, and wash it slightly in dilute chromic acid, then place it in the ordinary chromic acid mixture and proceed as described at page 15.

2nd Method. To show the peptic cells take fresh stomach and wash quickly, then plunge into pure methylated spirit.

3rd Method. Put the stomach in Muller's fluid unwashed, for 48 hours. Then cut narrow strips of the mucous membrane about half an inch long by one eighth of an inch wide and wash these in one-tenth per cent. osmic acid, then place them in half per cent. osmic acid from one to two hours to stain, then place them in one-sixth per cent. chromic acid and complete the hardening in the usual manner. The one-sixth per cent. chromic acid is here used without the addition of methylated spirit.

Some sections of stomach should be taken from the

different parts, pylorus, cardiac end, &c., and these should be stained in logwood ; those which are required to show the peptic cells should be stained in anilin blue.

Pyloric end of stomach with commencement of duodenum should be hardened in chromic acid mixture, or if the whole stomach has been hardened a portion showing the junction of these two parts should be cut with the microtome and double-stained, a few sections also should be stained with logwood. Examine these sections for the gradual change in the epithelium as the one organ passes into the other.

DUODENUM.

May be hardened in chromic acid mixture and sections stained with logwood.

Notice Brunner's glands cut in different sections, also goblet cells amongst the columnar epithelium and the fine non-striped muscle fibres running up from the muscularis mucosæ to the basement membrane.

ILEUM.

The whole of the intestine may be hardened in chromic acid mixture or in spirit mixture (page 17), it must not be much handled and should be first slightly washed in very weak solution of chromic acid. Sections are best cut with the freezing microtome and may be stained in a great many different ways.

In a section containing a portion of Peyer's glands, the treble staining process (page 40), may be used, and the result will be very good, as the Peyer's glands take on the green alone, without combining another colour with it, as all the other elements in this specimen do,

so that they are brought out as brilliant light green bodies.

ILIO-CŒCAL VALVE.

A section should be made through the ilio-cœcal valve with a little of the intestine on either side of it, and this should be trebly stained by the process mentioned at page 40. Some sections should, however, always be stained with logwood to compare with the others, as although double and treble staining differentiate the various tissues, logwood brings out the structural elements better than any other stain.

SOLITARY GLANDS.

Sections should be made through a piece of large intestine containing a solitary gland, and this will be well brought out by the treble-staining process

LIVER.

The liver may be prepared for examination in three ways :—

1. By the ordinary chromic acid mixture (page 15).
2. By dilute spirit (page 17).
3. By Muller's fluid (page 16).

1. *The Chromic Acid method.* The liver must be perfectly fresh and cut into small pieces about half an inch square, these should be placed at once in the fluid without washing. A large quantity of blood will exude from them after being in the hardening fluid a short time, and it will be necessary to change it in many cases at the end of 12 hours. It is a good plan to shake

the bottle containing the specimens in process of hardening occasionally; this must be done gently, so as to just alter their position, and when there is a quantity of sediment at the bottom of the bottle, and the fluid has lost its yellow colour and begins to look muddy, it is time to change it. Portions of liver require changing a little oftener at first than other normal structures.

2. The *spirit method* is used in the ordinary manner for liver, but the dilute spirit will generally require changing once before using the pure methylated spirit.

3. *Muller's Fluid.* This may be used when large portions of the organ are to be hardened and when time is no object.

When the material is well hardened by either of these processes, beautiful sections may be cut with the freezing microtome, and they show best when stained with logwood.

The intra-cellular and intra-nuclear network is seen very well in the cells of the liver, and makes an interesting object for a moderately high power. The specimens should be searched for bile ducts cut transversely, looking like minute triangular openings between the cells.

LUNG.

May be hardened in either chromic acid and spirit, or Muller's fluid, but to harden it well the fluid must be injected into the lung through the trachea. This is very easily done: the lung having been removed with a portion of the trachea attached, an ordinary brass syringe with ivory nozzle is filled with the hardening fluid, the nozzle inserted in the trachea, and the lungs gently distended with the fluid; when sufficiently full,

the trachea is tied and a weight attached. The lungs are then placed in a tall vessel containing the hardening fluid, which is changed as often as necessary.

TO SHOW THE EPITHELIUM OF THE ALVEOLI.

Inject through the trachea a $\frac{1}{3}$ per cent. solution of nitrate of silver, and then harden the lung by the spirit process (page 17), and make horizontal sections; these must be rather thick to get a correct idea of the epithelium as lining a cavity. These specimens will show the stomata between the epithelial cells.

Lung is best stained with logwood. Sections should be made through a bronchus and the small masses of ganglionic cells examined, these same sections may also be double or treble stained to differentiate the glands of the bronchi.

KIDNEY.

This organ is very well hardened in chromic acid mixture.

Remove the kidneys from a freshly killed animal, take one and divide it transversely into several pieces and place them in the fluid. The other may be divided longitudinally by one cut, and large kidneys may be hardened in chromic acid in this way. Sections may be cut by the freezing microtome, and are best stained in logwood, or double stained in picro-carmine and logwood (page 38). To show the minute structure of the cells in the collecting tubes, fine striation of epithelium, &c., Heidenhain's method is the best.

Cut the kidney into small pieces longitudinally in the direction of the pyramids, and place them in a 5 per

cent. solution of chromate of ammonia, from 24 to 48 hours in a stoppered bottle, then wash for several hours until no more colour comes away, changing the water several times, and place in dilute and then in strong spirit in the ordinary way (page 17).

Sections should be made both vertical and transverse of the cervical and medullary portion of the kidney, as well as large sections longitudinal and transverse of the whole organ; by this means the general structure of the organ and the arrangement of the tubes can be examined, while in the smaller sections, some of which should be mounted under thin covers, the minute structure can be studied; for this no stain succeeds so well as logwood.

Examine sections of the cortical part for the striation of the epithelial cells, and notice the imbrication in some parts.

BLADDER—URETER.

Cut into small pieces and harden in chromic acid mixture.

The bladder of ape makes very good material when hardened in chromic acid mixture, and transverse sections of the neck make very good specimens as they are not too large to mount whole.

TO SHOW GANGLIA OF BLADDER.

Remove the bladder, empty it, and place in $\frac{1}{2}$ per cent. solution of gold chloride 2 hours in the dark. Then place it in water well acidulated with acetic acid, until it has become swollen up to a good size, then preserve it in glycerine.

The gold will be seen deposited in small patches here and there on the surface, one of these is removed with a pair of curved scissors and mounted in glycerine.

GENITAL ORGANS—MALE.

TESTIS.

The best hardening fluid for the testis is undoubtedly the chromic acid mixture. The organ is cut into small pieces, or deep cuts made into it with a sharp razor, according to the size. It must not be washed and should be handled as little as possible.

Sections are made in different directions through the various parts, and it is advisable to make some large sections through the corpus Highmori and globus major to show the structure and relation of the different parts ; some of these sections will show the epididymis as well. These sections if at all large are very difficult to mount, as they break to pieces on the lifter, and it is often impossible to get them on to the slide whole ; in this case the best plan is to use the cover glass as a lifter, and clean it afterwards, when the Canada balsam has set.

Sections of testis are best stained in logwood, or double stained in picro-carmine and logwood (page 38).

Some of the thinnest sections should be mounted under ·003 cover glasses, as the developing spermatozoa form very interesting objects for the highest powers.

EPIDIDYMIS AND VAS DEFERENS

Are hardened in the same way as testis, and epididymis is generally hardened and cut with the testis.

Stain with logwood, or treble stain.

Examine the columnar epithelial cells of the epididymis with very long ciliary processes. With a good

object glass of moderate power these processes can be seen to be continuous with the longitudinal striation in the body of the cell, and can be traced by careful focussing through the striated line at the margin of the cells.

PROSTATE, GLANS, ETC.

These can all be hardened in chromic acid mixture in the usual manner. The glans may be placed in gold chloride for two hours and then hardened in spirit, after which longitudinal sections will show the nerve structures. The glans of a small animal hardened in this way and cut longitudinally, will show the difference between the epithelium of the mucous surface and that in the meatus and commencement of the urethra; it will also show large bundles of medullated nerves arranged in a peculiar manner, and many other things worth studying.

GENITAL ORGANS—FEMALE.

UTERUS, FALLOPIAN TUBES, VAGINA,

Can all be hardened in chromic acid mixture, and should not be washed unless absolutely necessary.

To examine the glands of the uterus it is better to use an animal that has borne young.

These organs may also be hardened whole in Muller's fluid or bichromate of potash. The sections are best stained in logwood.

OVARY

Is best hardened in chromic acid mixture, and should not be handled more than is absolutely necessary. The whole organ may also be hardened in Muller's fluid.

MAMMARY GLAND.

Cut small pieces and place in chromic acid mixture, when hardened stain with logwood.

PLACENTA.

The placenta of guinea-pig is the best for examination, and should be taken a little after half the period of gestation has passed. It may be prepared in chromic acid mixture or in spirit mixture. Vertical sections should be cut as thin as possible, and stained with logwood.

SPERMATOZOA.

The living spermatozoa of Triton cristatus make a most beautiful preparation and are readily procured.

Take a large male newt, which may be known by the serrated crest or fin along the back, and kill it, quickly remove the viscera, and the testes will be found, generally two or three each side, as small round bodies which cannot easily be mistaken. Take one of these and make a small cut in it, remove some of the milky fluid which exudes to a slide, and add a little salt solution or distilled water. Cover and examine. A power about $\frac{1}{8}$ will be required to see the spiral filament well.

A large number of spermatozoa will be seen in the field making slight lashing movements with the long filiform body, and on closer examination the filament will be seen in rapid movement; this movement commences at the elliptical body at the base of the head, and gives the idea at first sight that the filament is being poured out from it. After watching for some

little time, the movement will become slower, and it can then be seen that the filament is attached to the body by a membrane, and that it is waved rapidly from side to side; by carefully watching it as the motion gets slower and nearly stops, the membrane connecting the filament to the body can be clearly seen.

TO MAKE A PERMANENT PREPARATION OF NEWT'S SPERMATOZOA.

Place the testes in 5 per cent. solution of chromate of ammonia for 24 hours; wash until no colour comes away in distilled water, then divide one of the testes in two, and taking one half in a pair of forceps press the cut surface on a glass slide, a small quantity of fluid will adhere to the slide, to this add a small drop of glycerine and gently mix the two fluids. Cover and examine.

TO STAIN NEWT'S SPERMATOZOA.

After having washed away all traces of the chromate of ammonia, make an incision into the testis nearly dividing it, and place it in undiluted logwood stain for an hour or more, then wash away all superfluous stain and mount in glycerine. Spermatozoa may be double stained, but it is a very tedious process, as a little too long immersion in either stain spoils the whole process. The best way is, after washing off the superfluous logwood stain, to dip a portion of the testis in undiluted spirituous solution of rosein; a little must be mounted to see if the stain is deep enough, if not it must be dipped again.

It is possible in this way to get spermatozoa of newt or salamander with the long pointed head stained with

rosein, while all the other parts are stained with log-wood.

MAMMALIAN SPERMATOZOA.

To make preparations of mammalian spermatozoa, a little glycerine is placed in a watch-glass, and one or two drops of absolute alcohol added. A cut is then made into the globus major of a fresh testicle, and a little of the fluid removed on the point of a knife and placed on a slide; a small drop of the glycerine is then mixed with it, it is then covered and sealed with Hollis's glue.

HUMAN SPERMATOZOA.

A small drop of semen is mixed with glycerine, to which a little absolute alcohol has been added; it is then covered and sealed up. It is best in this case to use thin covers that have been measured, and to note their thickness on the label, as the human spermatozoon is so very minute it requires the highest powers to make out the filament.

SPECIAL SENSES.

INTERNAL EAR. COCHLEA.

The guinea-pig is the best animal, as the large tympanic bulla is easily exposed. Remove the periosteum from the bulla, and open it carefully with the point of a pair of straight scissors; as soon as a small opening has been made it can be enlarged, and the cochlea will be at once seen: as much of the surrounding bone must be removed as can be done without injury to the parts required, and it will be ready for preparation.

This may be done in several ways, but the two following are the best.

1. Place it at once in absolute alcohol, and let it remain 24 to 48 hours, then place it in $\frac{1}{10}$ per cent. solution of osmic acid for 24 hours. After this place it in a half per cent. solution of chromic acid, to which 1-2 drops of hydrochloric acid have been added. Let it remain in this until the bone is softened throughout.

2. Place the piece of bone in the ordinary chromic acid mixture for a week, and then remove it to $\frac{1}{2}$ per cent. chromic acid, to which 1-2 drops of hydrochloric acid have been added. Remove when the bone is softened.

When the bone is decalcified by either of these processes, it must be washed for several days, to remove the lime salt. It is then ready for cutting sections. In the case of the internal ear, sections are much better cut by hand, as the fine structures, such as the rods of Corti, are quite disarranged by freezing, and cutting with a microtome.

The bone must now be placed in gum solution for 24 hours, and then removed to spirit slightly diluted with water. If the spirit is too strong, the gum will form a substance like chalk, and quite as hard; this can, however, be softened by placing it in water. When the gum is sufficiently hardened by the spirit, it can be imbedded in wax mass, and sections cut by hand in the ordinary manner. These sections must be very gently handled, and should not be lifted with a needle but with a fine camel hair pencil. They are best stained in logwood. It is a good plan to stain the whole bone in logwood before placing it in the gum solution, but care must be taken that it is not too deeply stained. The undiluted logwood should be used, and it will require about 6 hours or more to stain it thoroughly.

Sections must be cut through the semi-circular canals and also through the cochlea, and the cochlea should be so embedded that sections will be cut through its whole length.

NASAL ORGAN.

The nasal organ is prepared in the same manner as the internal ear, and can be conveniently removed and hardened with it. Transverse sections should be made through the anterior part to show the membrane of the respiratory part, and transverse sections further back to show the olfactory membrane with its peculiar epithelium.

The septum of the nose should also be carefully removed from a specimen, and longitudinal sections made of it : if these sections are very carefully handled, they will show both the respiratory and olfactory epithelium very well. They are best stained in logwood and mounted in Canada balsam.

EYE.

The eye may be hardened in chromic acid mixture or in Muller's fluid. It must be removed without squeezing, and a few incisions made, it can then be hardened whole. When sufficiently hard, divide the eye longitudinally with a sharp razor.

RETINA.

The retina will be found lying on the inside of the posterior part of the eye, from which it may be gently detached by a spear-shaped knife, it may be then frozen

and sections cut; it is better, however, to stain it first, as thin sections are so transparent it is difficult to see them. When prepared in Muller's fluid the retina is very brittle, but in chromic acid mixture it is much tougher and can then be cut in strips and several frozen together in the microtome. Muller's fluid shows the nervous structure best, chromic acid the connective tissue. Good sections of the retina may be obtained by cutting the whole eye of the frog, and it may be double stained first.

To do this, first place the whole eye in a strong solution of rosanilin until it is deeply stained, then wash away the superfluous stain in methylated spirit, next place the eye for a short time in strong solution of iodine green and wash it well, soak in gum solution and freeze. By this means very good sections can be obtained, the granular layers having stained with the iodine green, the others with rosein.

They must be mounted in Canada balsam and should not be left long in spirit.

CORNEA.

The cornea may be removed from an eye hardened either in chromic acid mixture or in Muller's fluid, and sections made with the freezing microtome; they are best stained with logwood.

To demonstrate the Corneal Corpuscles and Nerves.

Remove the cornea from an animal just killed: this is done by cutting round the margin with fine curved scissors. Place the cornea in $\frac{1}{2}$ per cent. solution of gold chloride in the dark. Let it remain for from one

hour to one hour and a half for a guinea-pig, an hour and a half to two hours for a rabbit. Then place it in distilled water, which must be changed once or twice, for 24 to 36 hours exposed to the light, it will then have become a violet colour.

It is now placed in a mixture consisting of

Pure glycerine, 1 part.
Distilled water, 2 parts.

Let it remain in this for two or three days in the dark. It is then taken out, and gently washed and placed in a wide-mouthed vessel containing a filtered nearly saturated solution of tartaric acid. As it absorbs this liquid, the colour will become darker and it will sink to the bottom of the vessel. The vessel is now plunged into water at a temperature of 40° to 50° C. to such a depth that the two fluids will stand at the same height.

Sections may now be made with a very sharp razor by holding the cornea between the finger and thumb of the left hand. This requires great care, and cannot be done without a good deal of practice. The sections are mounted in glycerine and sealed with Hollis's glue.

Before cutting the sections, while the cornea is still in the distilled water, it is well to pass a camel's hair pencil gently over the surface, to remove the gold deposited there.

IRIS AND SCLEROTIC.

A portion of the eye containing part of the cornea, sclerotic, and iris may be cut out, and frozen. Sections of this stained with logwood, show the junction of the cornea and sclerotic, and the structure of the iris.

LENS.

In cutting sections of the whole eye of frog, good sections of the lens can be obtained. In the whole eye prepared by either of the methods given, the lens will be found hardened, when the eye is opened; and sections may be cut by the freezing microtome, but they generally break up if thin; enough, however, can be obtained to enable the student to examine into the structure.

PART II.

PRACTICAL PATHOLOGY.

CHAPTER I.

ON PREPARING AND MOUNTING PATHOLOGICAL SPECIMENS.

PATHOLOGICAL specimens can be hardened in the same manner as normal tissues by the processes already described in Part I. It is often necessary to find out at once what a tumour or new growth consists of, and for this purpose it is necessary to examine the fresh tissue. In some cases this is sufficiently hard to be frozen and sections made, while in others a small portion can be teased out on a slide and examined.

This may give a rough idea of what the morbid growth is composed, but for a thorough examination, and when it is desired to keep preparations of any disease, a complete process of hardening must be adopted, and sections cut and stained as in normal tissues, and for this purpose it is necessary to have the material as fresh as possible.

TO MAKE PERMANENT PREPARATIONS OF A CANCER IN A SHORT TIME.

This method may be applied to most of the sarcomata and carcinomata, and is valuable, as it can be used on portions of morbid tissue excised from the living body. Snip off a small portion of any morbid growth, such as cancer, with a pair of curved scissors. Place it in a mixture of dilute spirit (page 17) for 12 hours, then removed it to pure methylated spirit for 12 hours, and finally to absolute alcohol for 12 hours. It will then in all probability be fit to cut sections from. Imbed in wax mass and cut some sections by hand, stain with logwood and mount in Canada balsam.

Sections may also be cut with the microtome. The two ether microtomes, already mentioned, are the simplest to use for this purpose, but the Williams will do as well, although requiring more time, and the trouble of filling with ice and salt.

A portion of tissue, either hardened quickly, as above, or perfectly fresh, is placed on the plate of the microtome on a little mucilage, and covered with the same.

As soon as it is frozen, sections are cut in the ordinary manner, and the gum removed by warm distilled water; they are then stained with logwood, as already described for normal tissues. If they have been hardened in spirit, they must of course be soaked in water before being frozen, until all the spirit is removed.

Sections of fresh tissue must be mounted in glycerine and sealed.

When large masses are to be hardened, Muller's fluid or 2 per cent. bichromate of potash are necessary, and must be used in the manner described for normal tissue at page 16.

There are some morbid growths, such as medullary carcinoma, which cannot be dealt with in this way, and they must be hardened in the chromic acid mixture in the usual manner.

It is quite as important that pathological specimens should be properly hardened as normal tissues, but how seldom is this done. In the first place it is difficult to get the morbid tissues fresh enough, and yet they are often put on one side or at most placed in the lump in a small quantity of methylated spirit and water, and it is expected that good sections can then be prepared from them.

Nothing is more erroneous than this idea; the subject has been probably dead 24 hours at the least when the post-mortem is made, often longer, and in summer especially, this means utter ruin to such organs as the spleen. How important is it therefore that such organs should be put in the hardening medium at once when as fresh as possible. For this purpose a wide-mouthed bottle of Muller's fluid should be taken to every post-mortem examination, and small bits of any organ that may seem interesting on any account may be put in. A small paper label may be tied on and they can be separated afterwards.

They may with advantage be allowed to remain in the Muller's fluid for a week, they are then cut into small pieces and placed in the chromic acid mixture in separate bottles duly labelled.

Almost every morbid growth can be hardened in the chromic acid mixture (page 15), in the same manner as normal tissues. Brain and spinal cord, however, are better prepared in 2 per cent. solution of bichromate of potash (page 17).

ON DOUBLE AND TREBLE STAINING MORBID GROWTHS.

Some very good results will be obtained if the different double and treble-staining processes mentioned, as well as any others that may suggest themselves, are tried on different morbid growths.

For example, well hardened sections of rodent ulcer and epithelioma may be stained by the picro-carmine and logwood process. Other sections of the same material should then be stained with rosanilin and iodine green (see double staining) and the two compared. In this way, some definite result may be worked out, which by using other specimens of the same disease may be confirmed.

LARGE SECTIONS OF PATHOLOGICAL SPECIMENS.

If a large section is wanted o any morbid growth to show the distinction between the healthy and diseased parts, such as a section through a cancer and the side of the uterus from which it is growing, it is better to cut a moderately thick slice, say about a quarter of an inch, and harden it in chromic acid mixture, than to harden the whole mass in spirit, as it will be found a very difficult matter to cut large sections of spirit-hardened material; they become so hard that they make the knife jump, and the section is consequently uneven.

AMYLOID DEGENERATION

Is best hardened in chromic acid mixture, and in kidney especially is very well brought out by this method. To show the amyloid substance well it must be stained

a different colour to the surrounding tissue, and this may be done by several of the double-staining processes; as the anilin dyes seem to have a special affinity for amyloid degeneration, the following should be tried:

Rosanilin hydrochloride and iodine green (Hopkin & Williams).

Eosin and anilin blue.

A 1 per cent. solution of safranine gives a very good result. The sections must be deeply stained, and then washed out in spirit; they must not be allowed to remain too long in the spirit, or the whole of the colour will come out. They should remain until a peculiar brick-red colour has left them. They can then be further stained in methyl blue with a very good result, if this is carefully done.

HYDATIDS.

To make a preparation of hydatid cysts, take a portion of the wall of a large cyst and scrape off some of the gelatinous matter adhering to it. Place a little of this on a slide and tease it gently in a drop of glycerine, cover and examine. If there are any small cysts showing hooklets &c. well, seal it up with Hollis's glue.

The cysts may be first stained with one of the anilin dyes, and mounted in glycerine after all superfluous colour has been removed.

SHORT HISTORY OF THE MANNER IN WHICH A PORTION OF MORBID GROWTH IS PREPARED BY THE CHROMIC ACID METHOD.

1st day. Small pieces placed in chromic acid mixture (page 15).

2nd day. Fluid changed.

5th day. Fluid changed.
8th day. Fluid changed.
9th day. Spirit mixture (page 17).
10th day. Pure methylated spirit.
14th day. Plain water.
15th day. Mucilage.
16th day. Section cut, stained, and mounted.

ON SEALING UP PREPARATION JARS FOR THE MUSEUM.

Of all the various methods that have been tried to prevent the evaporation of spirit from the specimen jars, the following seems to be the best.

A glass cover must be cut to fit the jar, this can be done very quickly with a machine made by Sharratt & Newth, the top of the jar is then ground until it is perfectly even. This is very easily done on a flat stone, such as those used in paving the streets. Some water is placed on the stone, and the mouth of the jar is rubbed steadily on the stone until all uneven surfaces are ground off, a few minutes will do this. Fine emery powder will assist the process, but is not necessary, and coarse emery must not be used as it will chip off the edges of the jar. Care is requisite in grinding down a large jar, as if it is allowed to jump it will soon crack.

To make the cement, a quantity of sheet gelatine is cut into small pieces, and covered with warm water in a porcelain dish on a water bath, it is stirred with a glass rod until dissolved, and then a small quantity of glacial acetic acid added until it becomes distinctly sour to the taste.

It is then poured into a wide-mouthed bottle, and is ready for use.

TO SEAL UP A PREPARATION JAR.

Place the cover on an iron plate, over a gas burner and warm it.

Cut with a file small notches in the top of the jar for the strings which hold up the preparation to lie in; thoroughly dry the top; for this purpose a piece of heated iron is drawn over it two or three times.

The cement having been made fluid in a water bath is applied to the top with a small brush, the cover is then placed in position and gently pressed down. A small weight in some cases may be placed on it. The jar is then put on one side for twenty-four hours. The space between the edges of the cover and jar is then filled in with warm gelatine solution, and when this is quite hard a ring of Brunswick black or Asphalt Varnish is painted on it. In sudden changes of temperature from warm to very cold it is advisable not to mount specimens, as the application of heat will crack a large number of the jars. Should the spirit after a length of time become low, it may be renewed without removing the cover. Two small holes should be drilled in the cover with an American drill moistened with turpentine, a small funnel is then drawn out very fine in the gas blow-pipe, and inserted in one of the holes and the jar filled up. The holes are sealed with the gelatine.

MODE OF PRESERVING OPHTHALMIC SPECIMENS.*

Mode of Preparing and Mounting.—The following are the stages of the process:—

1. The eye is placed immediately after excision, un-

* By Priestley Smith, *Ophthalmic Review*, March, 1883.

opened, in Muller's fluid for about three weeks, light being carefully excluded. It is well to change the fluid every two or three days, otherwise the specimen may be permanently stained; this happens all the more readily if light be not excluded. The fluid consists of

Bichromate of Potash	...		1 part.
Sulphate of Soda ...	...		1 ,,
Water ...	...	...	100 ,,

2. It is then wrapped in a piece of gutta-percha membrane, the surface of which has been greased to prevent adhesion, and *frozen solid* by immersion in a vessel containing a mixture of ice and salt. The vessel should have a hole at the bottom, so that water may drain away; a flower-pot answers well. To freeze the eyeball solid takes not less than half an hour; a valuable specimen may be spoiled by disturbance of the internal parts if cut open before it is solid throughout.

3. When frozen it is divided in the required direction by means of a sharp table knife. A thicker blade such as a razor, goes through the frozen globe with difficulty. If the exact position of the section is of consequence the points through which it should pass should be marked with a spot of ink before freezing.

4. The bisected specimen is placed in a 5 per cent. solution of chloral-hydrate in order to remove the colour of the Muller's fluid, the solution being changed every two or three days until it is no longer discoloured.

5. It is then placed successively in glycerine solutions, 10 per cent., 25 per cent., and 50 per cent., remaining in each for twenty-four hours or more. This process is necessary in order to prevent shrinking of the tissues when the specimen is placed in the jelly.

6. It is then mounted. A specimen-jar being filled

with melted jelly, the half-eye is placed in it, the concavity upwards. When every interstice is filled it is turned over, care being taken to avoid the imprisonment of an air-bubble, and held, by means of a needle, in contact with the bottom of the jar. When the jelly is coagulated the jar is closed by glueing a disc of white cardboard upon its open end. The cardboard forms a white background to the specimen; it is not in contact with the jelly.

The jelly is made according to the following formula :—

French Gelatine	...	...	1 part.
Glycerine ...	...	...	6 parts.
Water ...	...	...	6 „

Soak the gelatine in the water until it is swollen; heat it, and add the glycerine; add a trace of carbolic acid; filter, while hot, through white blotting paper.

The strongest and most colourless gelatine is that made by Coignet & Co., of Paris, obtainable in packets, and known as the "gold label" variety. The specimen jars are manufactured expressly by Messrs. F. & C. Osler, of Broad Street, Birmingham. The gelatine may be obtained from Shew & Co., Newman Street, Oxford Street.

CHAPTER II.

ON BACTERIA.

ON THE CULTIVATION OF BACILLUS ANTHRACIS.

THE following is Dr. Klein's method of cultivation, published in the Report of the Local Government Board, 1881–2 :—

Method of Cultivation Employed. As nourishing fluid I have employed broth prepared from fresh pork. About a pound and a half to two pounds of pork are boiled in water for an hour or so, down to about two pints of fluid. The fat scum is removed, and the fluid, provided the pork employed has been lean, filters tolerably clear through filter paper. To obtain it, however, perfectly limpid, the broth is cleared à la cuisine with egg-albumen and then filtered. The filtrate, which will be spoken of as "the pork broth," is of a neutral or faintly acid reaction; in this latter case a sufficient amount of carbonate of sodium is added in order to make it neutral; it is then placed in long-necked flasks, which are plugged with cotton wool.

In all cases, and I wish to state this once for all, where a cotton-wool plug is spoken of, whether in connection with a flask or test-tube, it will be understood that a cotton-wool plug of about two inches in length is meant, in some instances two plugs, one above the other, each about an inch long, being used. The cotton wool, the flasks, beakers, filters, filter paper, test-tubes, and all vessels used, are invariably disinfected by exposing them

for several hours, generally repeated several times, to a temperature varying between 140° to 150° C. To use cotton wool disinfected by prolonged (for several days or weeks) steeping in absolute alcohol, or concentrated carbolic acid solution, is not absolutely reliable. Over-heating the cotton wool in an air chamber to the above temperature till singed has proved invariably and absolutely safe for all cultivations; I have had, to my regret, failures in my cultivations which could be referred to cotton wool soaked in concentrated carbolic acid solution even for months. The same is to be said of the flasks and test-tubes used. No amount of cleaning, even with strong acid, is to be relied on; nothing but over-heating gives reliance. I at first always used to heat the vessel well all round, over the open flame of a Fletcher's burner, almost till the glass becomes glowing, and soon after, when the glass is still hot, but not to do more than just singe the cotton wool, I place in its neck the cotton-wool plug, this having previously been over-heated in the air chamber. But lately I have over-heated the vessels in the air chamber to about 140° to 150° C. for several hours, several times repeated, having previously cleaned them with distilled water, and dried them as far as possible; and I have found this perfectly sufficient to disinfect them thoroughly. It cannot be too strongly insisted on with Koch that the flasks and test-tubes, and especially the cotton wool used as plugs for the vessels, should be thoroughly sterilized by over-heating, for cultivations are as often contaminated by this not being properly carried out as by the non-sterility of the nourishing fluids or the accidental entrance of organisms from the air.

The filtered nourishing fluid (pork broth) having been placed in a clean flask, plugged, as above stated, with

long and clean cotton wool, is boiled from about ten to fifteen minutes. I never fill the flask to more than half its volume with the broth, in order to avoid the fluid rising too high during boiling, and thus wetting the cotton-wool plug. This, although not necessarily fatal, owing to the sterility of the cotton wool, nevertheless I always avoid in this and other cases, for the sake of cleanliness, and to avoid all possible contamination. Immediately before turning off the flame of the burner, and while the fluid is still boiling, I place over the mouth of the flask a cotton-wool cap, and keep this pressed over the mouth and upper part of the neck of the flask by an inverted beaker pushed firmly over it. The flask is then placed in an incubator, and kept there at a temperature of about 32° to 35° C. After two or three days the flask, plugged, but without the cotton-wool cap, is again placed over the gas-flame, and the broth boiled for five to ten minutes. While still boiling, the cotton-wool cap and beaker are placed over the mouth and neck, and the flame is turned off. Such a flask with broth may now be considered absolutely sterile; it may be kept in the incubator for weeks and months—it will always remain absolutely limpid and free from any organisms. Such broth will in the future be always spoken of as "sterile pork broth."

This broth I use either as such, *i.e.*, as pure broth, or in combination with gelatine, as "gelatine pork," in order to have, as recommended by Koch, a nourishing material, not of fluid but of solid consistency. I consider, with many others, this method of Koch's, viz., of using gelatine as an admixture to a nourishing fluid, and thus converting it into a solid state, a very great advance indeed in the methods of cultivating bacteria, especially in securing pure cultivations not

contaminated accidentally, for then the sowing of a particular species of bacterium is possible in a particular spot or spots, the growth and progress can be easily watched and controlled, and accidental contaminations can be readily recognized; but I shall show below that it is quite possible also without the gelatine admixture after the method I use to be almost absolutely guarded from accidental contamination, *i.e.*, to have pure cultivations. Koch has very minutely described the advantages of the gelatine method and his *modus procedendi*, and he has given numerous photographic illustrations of various species of bacterium in pure cultivations effected by his gelatine method.

Koch recommends, in order to solidify the nourishing material (in his case it was a solution of Liebig's meat extract), to mix with it purified and well sterilized and neutralized gelatine solution in such a proportion that the gelatine would form 2 to 3 per cent. Such a nourishing mixture is solid at ordinary temperatures, and represents an excellent soil for sowing on or in it the desired species of bacterium in dots or lines; kept in flat glass dishes or slides the examination with the microscope can be easily carried out from time to time, and it can easily be ascertained how and whether the sown species is making progress, and accidental contaminations can thus be easily detected and removed, all growths, owing to the solid state of the nourishing material, being naturally limited to the spot or line on which the bacterium has been sown. It is necessary to keep the dish or glass in a chamber (under a bell-jar) saturated with moisture. This is, in short, the essence of Koch's method. He maintains that such a gelatine material remains solid at a temperature of 20° to 25° C., sufficiently high for the growth of all species of bacteria.

All this sounds very excellent, but when one comes to work with it practically one finds that everything is not as perfect and excellent as one imagines at first.

As is well known from the researches of Brefeld, Grawitz, Wernich, and others, nourishing material in a solid state, such as gelatine, boiled potato, bread, paste, &c., has been used for the sake of obtaining pure cultivations, and for the sake of easily watching and keeping under control the progress and growth of particular organisms, *e.g.* Penicillium, Aspergillus, *Micrococcus prodigiosus*, &c.; but most of these observations were carried on at ordinary temperatures. Koch, however, recommends it, after many observations, in the above form for pure cultivations, even in the incubator, at 20° to 25° C. for all species of bacteria (Micrococcus, *Bacterium termo*, and various species of baccilli, &c.).

The first difficulty one has to overcome is to obtain a sterile and neutral clear and limpid gelatine solution. I have found best answering my purpose a gelatine solution prepared in the following manner: one part of "gold-label gelatine" (the tablets in which it is sold being cut up into small strips) is soaked overnight in six parts of cold water, it is then dissolved on the water bath; this solution has a slightly acid reaction; to it is added carbonate of sodium just sufficient to give it a neutral reaction. While quite hot it is filtered through filter paper once or twice. (It must be borne in mind that the filter paper, the vessels receiving the solid gelatine or the filtrate, and all other vessels subsequently used for its reception, are perfectly disinfected by overheating them.) The process of filtering is carried out by using hot filters and filter paper, keeping up the warmth by placing at opposite sides, as close to the filter as practicable, Bunsen burners. The filtrate is

tolerably clear, but can be obtained perfectly clear by adding to it after neutralization egg albumen, and then boiling it for several minutes. In this latter case it may be filtered through calico or flannel previously disinfected. To the filtered gelatine are then added three parts (not three times its volume) of the above pork broth, so that we have now altogether one part of solid gelatine, six parts of water, and three parts of pork broth, which would be equal to one part of solid gelatine in nine parts of fluid, or $11\frac{1}{9}$ per centum. This mixture is placed in several sterilized flasks, closed well with long and thoroughly sterilized cotton-wool plugs, and is subjected to boiling from 5 to 10 minutes. While still boiling, and just before removing from the flame, the mouth of the flask is covered with a cotton-wool cap, and a beaker is inverted over it. The flasks are then placed in the incubator and kept there at 32° to 35° C. for twenty-four hours, after which they are again subjected to boiling for about five minutes. This I have found to be sufficient to keep them sterile for ever after. This mixture, which I will speak of as "sterile gelatine pork," remains, even in the smallest quantity, solid up to a temperature of 25° C., a temperature generally sufficiently high for the growth of bacteria.

I will now describe the method of using the above nourishing material for the special cultivation of the *Bacillus anthracis.*

(A.) A number of disinfected test-tubes and small flasks are used, the latter of the capacity of an ounce or so, plugged with disinfected cotton-wool, the plug lifted, and each charged as rapidly as possible with a small quantity of the nourishing material from the stock flask, and then plugged with cotton-wool. In the case of the gelatine pork, this is of course first liquefied over

the flame. The stock flask, if not emptied by this process of charging, is subjected to boiling from five to ten minutes. When charged and plugged each test-tube and small flask is subjected to boiling for a few minutes; the boiling is effected over a small flame in order to prevent the over-boiling; this is not so much to be feared in the case of the flasks as in that of the test tubes. Thorough boiling for once is generally sufficient to destroy every organism that may have accidentally entered during the process of charging. Kept for an indefinite time in the incubator at 32° to 35° C. the fluid in them remains bright and clear.

(B.) Glass cells of exactly the same nature as those that were of so great use to me in my research on the pneumo-enteritis of the pig (see Local Government Board Reports for 1877, p. 210), in the majority of instances without any addition, in some with the addition of a thin glass tube cemented to the glass slide and leading into the cell; the outer opening of this glass tube is plugged with cotton-wool. This tube was chiefly added with the view of facilitating the formation of spores, but as a rule I found, *cæteris paribus*, if the other conditions for the spore formation are present, the amount of air present in the glass cell was sufficiently large to allow of spore formation.

As in my former work so also now, I use olive oil to fix the cover glass over the glass ring forming the sides of the cells. The cover glass before being used is well heated over the flame. A small quantity of the nourishing fluid (pure pork broth or liquefied gelatine pork) is withdrawn from the stock flask by a freshly drawn-out small capillary pipette; it is effected in this manner: the cotton-wool plug of the stock flask is drawn up for about half its length, and the one end of the pipette

being drawn out into a long capillary tube is gradually pierced through the remaining half length of the plug and pushed down till it reaches the fluid; the pipette is filled and withdrawn, and the plug is again pushed down into its previous position. By this means absolutely no access is allowed to particles from the air into the stock flask, and at the same time the capillary tube, while being pushed through the cotton-wool plug, is cleaned from accidentally adhering particles. It must be borne in mind that for the above purpose the cotton-wool must have been well sterilized by heat, because if not so, the nourishing material in the stock flask is sure to become contaminated by impurities adhering to the cotton-wool fibres, some of these being pushed down as well as carried down into the fluid by the capillary tube. From this pipette a drop is quickly deposited in the centre of the cover glass, and this is inverted and fixed on the ring of the glass cell, a drop of distilled water having been previously placed at the bottom of the cell at the edge. The cell is now "charged" and ready to receive the organism that is to be cultivated in the drop of nourishing material attached to the centre of the lower surface of the cover glass. The process of charging the test tubes and flasks, as well as the glass cells, being carried out in the air, is of course subjected to the complication of a contamination with air organisms. In the case of the test tubes and flasks this is remedied by subsequent boiling of the charged and plugged vessels; but in the case of the glass cells a sterilization after charging is for obvious reasons impossible, and it is therefore necessary to take one's chance, so to speak, of having a number of failures owing to accidental contamination. And it is this very point, viz. the chance of contamination with air organisms, which

makes the Koch's method, as recommended by him, impracticable in the case of many cultivations, as I shall have to point out below in detail.

It depends very much on the place and season where and when the charging is carried out, as regards the accidental contamination with air organisms. I have made some comparative studies on these points, and I think it worth while to enter here more fully into them.

At first, when working at the laboratory of St. Bartholomew's Hospital Medical School, I charged my test tubes from my stock flask under carbolic acid spray, the carbolic acid being of the strength of about 5 to 6 per cent. From my notebook I gather that in one series I charged sixteen test tubes carefully under the carbolic acid spray, and placed them in the incubator at about 35° C. Of these test tubes one went bad in the course of twenty-four hours, at which time it became turbid owing to the presence of actively moving bacilli. In another series of fourteen test tubes two went bad. In a third series of twenty-two test tubes every one went bad, although the method of charging under the carbolic acid spray was the same as in the other cases; but the conditions of the atmosphere were not the same. While I had tolerably good results in July and August I had very bad results in October, and my failures, both in preserving sterile my stock fluids and my test tubes charged with them, became during this month so numerous and persistent that I had to give up work altogether for this period. To have cultivations exposed to the air and not afterwards sterilized, as is almost the general rule in Koch's method of gelatine cultures, and to keep them pure, was altogether out of the question. The cause of these universal and unconditional failures was

not far to seek. During October we had a good deal of dry weather with strong winds, and the laboratory in which I worked faces Smithfield hay market, from whence a good deal of dust was blown. This dust contained an enormous number of spores, especially of bacilli, as was proved not only by direct observation, but also by the fact that every kind of nourishing fluid, Cohn's nourishing fluid, hay infusion, beef broth, mutton broth, pork broth, &c., previously sterile, when exposed to the air on such windy days for a second became very difficult of sterilization; boiling for ten minutes and sometimes fifteen minutes, or even more, did not produce sterilization. After forty-eight hours' incubation the fluid was invariably swarming with bacilli.

During July, August, and September, when days were tolerably still, especially on rainy days, and there were no high winds, test tubes containing sterile nourishing fluid could be kept open, *i.e.* the cotton-wool plug could be altogether removed under carbolic-acid spray for several seconds, and without being subjected to boiling after this remained sterile at a temperature of 35° C., only a relatively small percentage, varying from five to seven, being lost by air organisms. This is not at all an unsatisfactory result, considering that the laboratory faced the hay market, and considering how easily a contamination could occur under these conditions. But at a time with high winds the contamination was so serious that even prolonged boiling after exposure did not sterilise. This is not to be wondered at if we remember that fluids containing hay bacillus spores and some other bacillus require for absolute sterilization boiling extending up to and even over half an hour (see Cohn's 'Beiträge,' ii. Bnd., ii. Heft). The results obtained sub-

sequently, when resuming my work, not in the previous locality, but in the laboratory of the Brown Institution, near Vauxhall, situated in a less contaminated atmosphere, were very much more satisfactory. Comparative experiments which I here made showed that exposing to the air for half a minute sterile nourishing fluids contained in test tubes during windy weather yielded about 50 per cent. failures, while exposing them to air under the carbolic acid spray yielded no failures in one series, it yielded 5 per cent. failures in another series.

As I mentioned above, I charge my test tubes rapidly either with pure pork broth or with liquefied gelatine pork without spray, and then boil them well for a few minutes, and doing this in the laboratory of the Brown Institution I find it sufficient to thoroughly sterilise the fluids.

The next important step in the cultivation of bacteria in the nourishing material hitherto described, as contained in the test tubes or glass cells, is the inoculation of these materials with the organisms it is desired to grow, *i.e.*, the process of sowing. It is, of course, obvious that if it is desired to cultivate a single species of organism, it is necessary to sow a single species, and to prevent contamination with air organisms, the nourishing fluid itself being sterile. With reference to the first, it is necessary to be certain that the material containing the seed and to be transferred into the nourishing material contains no other but the desired species. This is, however, not always a simple matter. It is simple enough in the following cases :—If I transfer to my nourishing material a droplet of blood taken from the heart or the spleen of an animal just dead or dying of anthrax, I am certain to have no other organism in the blood except the *Bacillus anthracis;* or if I have an artificial cultiva-

tion of *Bacillus anthracis* which from certain definite naked-eye appearances, and still more from microscopic examination of anilin-stained specimens, I can pronounce with certainty to be a pure cultivation of *Bacillus anthracis*, I shall be certain that I shall again, *cæteris paribus*, obtain a pure cultivation, if sowing from this cultivation. Again, if I take an infusion of hay in which fermentation produced by the hay bacillus has been completed—that is to say, in which the bacillus has passed its whole cycle and has yielded an abundant crop of spores forming a fine brown precipitate at the bottom of the infusion—and if I boil this infusion for several minutes, I shall be sure to destroy everything living except the spores of the hay bacillus, and if I sow from this so-boiled infusion, I shall have the satisfaction of finding that the new growth contains only hay bacillus.

The above modified use of Koch's method, viz., charging the covering glass of the glass cell with a drop of liquefied gelatine nourishing fluid, and when this has become solidified again inoculating it in one or two straight lines with matter containing the bacteria to be sown, *i.e.*, to dip a needle previously heated, or the end of a freshly drawn-out capillary tube into the fluid containing the seeds, and then to draw this needle or the capillary tube quickly across the surface of the drop of gelatine nourishing material once or twice ; this method, I say, is invaluable for the study of the gradual changes those bacteria undergo when subjected to incubation, the manner in which they multiply ; further, to ascertain whether the desired organism has been sown, and whether only one kind of organism or several are growing in the nourishing material ; for the glass-cell specimen can be easily examined, even with high powers of

the microscope from time to time, without in the least disturbing the growth. Koch, in his paper above quoted, has minutely described all these advantages, and therefore I need not further enter into this part of the subject, as I have no doubt it must be obvious to every one who has the slightest acquaintaince with artificial cultivations of bacteria.

If you have sown in this manner a particular organism well known to you, it is of course easily ascertained on microscopic examination immediately after, whether the same is present in any part of the line you have drawn over the gelatine drop in the above glass-cell specimen with your needle or capillary tube. Thus, inoculating the gelatine drop with the *Bacillus anthracis* or with its spores, or the spores of hay bacillus, with sarcinæ, with torulæ, with *Micrococcus prodigiosus*, &c., you can at once find these seeds in the streak you have drawn on the gelatine drop ; according to the number of seeds present in the material to be sown there will be more or less numerous seed in that streak. If in addition to this you have sown only one species of those named, any accidental contamination will soon be detected under the microscope in the gelatine drop, say after a day or two, or longer.

But supposing you are sowing a material of which you do not know whether it contains any organism, or, if so, what kind of organism, the case is altogether different, and the value of this method is not obvious ; on the contrary, may lead to serious errors ; in this way : the inoculation of the solidified gelatine nourishing material, whether in my glass-cell specimens or after Koch's plan, on glass slides or flat dishes, must take place in the air, and there is no means to prevent contamination with air organisms. Under ordinary circumstances, and working

quickly, the chances of such contamination are not very great, but are, nevertheless, objectionable. Now, supposing that you inoculate your gelatine in several specimens with the material to be tested for organisms, you may find after a day or two or more of incubation that in one or more of the specimens in the streak you have drawn there is no growth whatever of any organism, but outside it at other points an organism or several organisms begin to grow, you will justly say that all these organisms are accidental contaminations, air organisms; but if you found in the streak one species or more growing you cannot conclude from this that you have transferred this or these species from your original material, because your moist needle-point or capillary glass tube may have caught these seeds while passing through the air; and this has actually happened to me, not once, but repeatedly. I have several instances in which I have sown, or meant to have sown, in one streak over the gelatine drop of my cell specimen a particular species of bacillus, and to my great annoyance I found, after several days' incubation, in that very streak growing three different species of organisms, viz., one kind of micrococcus and two different species of bacilli. In another instance I wished to test a fluid for the presence of an organism or organisms, consequently I sowed it on the gelatine in several of my glass cells, and I obtained in the streak drawn over the gelatine drop two species of organisms, a micrococcus and a bacillus; but as I ascertained with a more precise method, the fluid contained no organism whatever. These facts, it must be conceded, prove that the method of Koch, although of great value in certain cases, is less to be recommended in others, and therefore does not deserve that unqualified praise which its author accords to it, saying as

much as that this is the only method after which cultivations of micro-organisms are to be carried on. I shall presently show that there is a more reliable method (provided the question is one of transferring one definite organism from one fluid into a vessel containing the nourishing material), a method in which the chances of contamination are less and the method also, for other reasons, more practicable.

The method of inoculation of the nourishing material which I at first used was under the protection of the carbolic-acid spray: a freshly drawn-out capillary pipette is dipped into the material to be sown, the cotton-wool plug of the test-tube or flask containing the nourishing material is lifted under carbolic-acid spray on one side just sufficient to admit the end of the capillary pipette; this being done the plug is again closed over the mouth, the capillary tube is pushed down into the nourishing fluid and then quickly withdrawn, and the plug completely replaced. In this manner I have been very successful in inoculating, without contamination with air organisms, nourishing fluids with the special organism desired to be sown.

But this method is unpleasant, as the spray prevents one from seeing easily the capillary tube while being pushed down into the test-tube. Although I used this method a good deal, I have nevertheless recently employed a much simpler method, which yields as good if not better results. In the carbolic-acid spray the chances of contamination with air organisms are small, as I have above stated, and when a contamination with them occurs it is probably through the spray catching them and carrying them into the test-tubes; but it must be obvious that this is really only a remote chance, con-

sidering that in my cases I only momentarily lift one side of the plug sufficient to admit the end of a capillary tube.

The best and most practicable method which I am now in the habit of using, and which is almost absolutely safe against accidental contamination, is this: the cotton-wool plug of the test-tube or flask containing the nourishing material is pulled out for about half its length; a capillary pipette having been charged with the fluid to be sown is then gradually and carefully pierced through the remaining part of the cotton wool (thereby clearing itself of adhering particles), introducing it between the plug and the sides of the vessel; it is then pushed down into the nourishing material and a trace of the seed fluid emptied into the former. The capillary pipette is quickly withdrawn, and the cotton-wool plug pushed down into its old position. If the nourishing material is gelatine pork, it is of course easily possible at will to deposit the seed from the capillary tube either on the free surface or in the depth. If the seed fluid is to be obtained from an artficial cultivation contained in a test-tube or flask, it is withdrawn with a freshly made capillary pipette in exactly the same manner as the seed material is introduced into the new cultivation just described. It must be borne in mind that for the success of this method it is imperative, in a greater degree than in the other previously mentioned methods, that the cotton-wool plug is thoroughly sterilized. For it is obvious that if this is not the case, by the piercing of the cotton-wool plug with the capillary pipette, wool fibres are always carried down into the nourishing material, and if these are not thoroughly sterilized a contamination of the latter must inevitably follow.

I had charged twelve test-tubes with pork broth, and

had them well plugged with cotton-wool, well boiled on two successive days, and placed in the incubator at 32° to 35° C.; they were kept there for two weeks, and remained perfectly limpid and sterile. I then inoculated six of them in the above manner with *Bacillus anthracis* of an artificial cultivation, viz., introducing the bacilli by piercing the capillary tube containing them through the cotton-wool plug. After twenty-four hours all showed signs of accidental contamination. I remembered that I had kept the test-tubes for several hours, and at two successive days, at 140° to 150° C.; but the cotton-wool had been tightly compressed in a beaker, and exposed only for about an hour to a temperature of about 120° C. From the remaining six test-tubes I removed the plugs of this cotton-wool, and closed them with fresh plugs of thoroughly sterilized cotton-wool. They were well boiled and kept in the incubator for several days; as they remained quite limpid, they were inoculated after the same manner and with the *Bacillus anthracis* of the same cultivation as in the case of the first six test-tubes; the result was completely satisfactory; no accidental contamination occurred. From this it is clear that the test-tubes and the nourishing material were sterile in both instances, and also the bacillus to be sown was the same, and in a pure state in both cases, but in the first the cotton-wool was at fault, hence the accidental contamination introduced into the nourishing material.

As a rule, in cases where the naked-eye appearances do not and cannot give indications of the actual state of the cultivation, *i.e.*, whether pure or not, as is the case in most cultivations of bacteria, except, perhaps, of *Bacillus anthracis*, I have employed both methods, *i.e.*, I cultivated it in the test-tube or flask, and at the same time controlled it under the microscope, by cultivating

in the above glass cell a specimen in a drop of solid gelatine nourishing material.

In the cultivations of *Bacillus anthracis* in the above-named neutral pork broth in test-tubes or small or large flasks with which I worked, after three or four or more days' incubation, even at a temperature so low as 20° to 25° C., a beautiful whitish crop of the bacilli is visible at the bottom of the vessel in the shape of a fluffy, nebulous, more or less filamentous mass, as incubation proceeds gradually extending into the further layers of the fluid, this latter being tolerably bright and limpid.

DR. KOCH'S METHOD OF CULTIVATING THE TUBERCLE-BACILLUS.

(Translated by Dr. Klein, *Nature*, May 4, 1882.)

Pure serum of blood of sheep or cattle is sterilized by keeping it exposed in test-tubes plugged with cotton-wool, for six days daily for one hour, to a temperature of 58 centigrades. After this the serum is heated for several hours up to a temperature of 65 centigrades; by this it is transformed into a solid perfectly transparent mass, well adapted for the cultivation of the tubercle-bacilli. Such serum inoculated on its surface under special precautions with tubercular matter of any source—tuberculosis of man or animal, spontaneous or artificially produced, and kept at a temperature of 37 or 38 centigrades (*i.e.* about blood-heat) for over a week, becomes gradually covered with peculiar dry scaly masses; these masses are the colonies of the specific tubercle-bacillus.

A minute particle of this crop is used for establishing a second similar cultivation, this again for a third, and so on. Tubercle-bacilli obtained in this manner, after

several successive generations, prove as effective in inoculating animals with typical tuberculosis as fresh tubercular matter.

All animals susceptible to the malady that Koch inoculated with these artificially cultivated bacilli, became invariably affected with the disease; not one escaped; while other similar animals kept under precisely the same conditions, except that they did not receive any tubercle-bacilli, remained perfectly healthy.

It is important to notice that the tubercle-bacilli require for their growth and multiplication a temperature of at least 30 centigrades, and, consequently, they are limited to the animal body, unlike the bacillus that produces splenic fever or anthrax, which is capable of multiplication at ordinary temperatures, as low as 20 centigrades, and even less.

CHAPTER III.

ON STAINING BACTERIA.

THE following method is applicable to all Bacteria and Micrococci, with the exception of those of tubercle and leprosy :—

A ½ or 1 per cent. solution of either Gentian violet or Spiller's purple is made in water. The fluid containing the Bacteria is dried on a cover-glass, a little of the staining solution is poured into a watch-glass, and the cover-glass placed on it, with the dried material downwards. It is then left for some time. This will depend on the material to be stained, and can be easily found out with a little practice. When thoroughly stained, the cover-glass is washed in methylated spirit until all superfluous colour is removed, taking care that the film containing the Bacteria is not washed off. The cover-glass is then drained by holding it on its edge on filter-paper, and it is allowed to dry. It is then mounted in the usual manner with Canada balsam solution.

TO STAIN SECTIONS OF TISSUE CONTAINING BACTERIA.

Place them in either of the above-mentioned solutions and allow them to remain for some hours.

When deeply stained, wash in water to remove the excess of the stain, and then lay them out flat in methylated spirit until no more colour comes away. Transfer

to absolute alcohol, and then oil of cloves, and mount in Canada balsam.

ON STAINING THE TUBERCLE-BACILLUS.

DR. KOCH'S METHOD.

(From *Brit. Med. Journal*, May 13, 1882.)

One cubic centimetre of a concentrated alcoholic solution of methylen blue is mixed with 200 cubic centimetres of distilled water; to this is added two cubic centimetres of a 10 per cent. solution of caustic potash, the fluid being well shaken. The material to be stained is kept in this solution for from twenty to twenty-four hours, or, if at the temperature of 40° Cent. (104° Fahr.), for a half to one hour. The cover-glasses on which broken down portions of tubercle have been dried, are then placed for two minutes in a filtered concentrated watery solution of Vesuvin. As a result, the methylen blue is displaced by the vesuvin from everything but the tubercle-bacilli; so that, after washing in distilled water, the bacilli stand out as blue rods on a brown ground. Sections are treated similarly; after having lain for twenty-four hours in the methylen blue they are transferred to the filtered concentrated watery solution of vesuvin for fifteen to twenty minutes, then they are placed in distilled water till no more of the stain comes out, and afterwards in alcohol, oil of cloves, and Canada balsam. Here, also, the nuclei are stained brown, while the tubercle-bacilli appear as delicate blue rods. All the other forms of bacteria which Dr. Koch has as yet examined in this way are stained brown, with the exception of the bacilli found in leprosy, which also retain the methylen blue in

preference to the vesuvin. These bacilli may also be stained by other aniline dyes if the solution be made alkaline by the addition of caustic potash or soda.

DR. EHRLICH'S METHOD.

(From *Brit. Med. Journal*, June 17, 1882.)

The sputum is spread in a thin layer on a cover-glass, and dried. In order to fix the albumen, the cover-glasses are kept at a temperature of 100° to 110° Cent. (212° to 230° Fahr.) for an hour; or, in practice, it is sufficient to pass them three or four times through a gas flame. The staining solution is prepared as follows:— About five cubic centimetres of pure aniline are added to 100 cubic centimetres of distilled water, well shaken, and then filtered through a moistened filter. To this mixture, a saturated alcoholic solution of fuchsine, methyl-violet, or gentian-violet, is added till precipitation commences. The cover-glass is allowed to float on this, with the side on which the sputum has been spread directed downwards, for a quarter to half an hour. It is then washed for a few seconds in a strong solution of nitric acid (one part of commercial nitric acid to two parts of distilled water), and afterwards in distilled water. In this way, the stain is extracted from everything but the tubercle-bacilli. The ground substance may be stained brown (if the bacilli have been stained violet), or blue (if the bacilli have been stained red), by way of contrast to the bacilli.

WEIGERT'S MODIFICATION OF DR. EHRLICH'S METHOD.

(From *Brit. Med. Journal*, March 17, 1883.)

Take of a saturated watery solution of anilin, 100 parts; of a saturated alcoholic solution of the basic anilin dye (methyl-violet, gentian-violet, fuchsin), eleven parts. Mix and filter before use. Rapid staining is obtained by warming the solution. The specimens are then decolorised by immersion in nitric acid (one part to two of water), and stained in a suitable contrast dye. Very delicate sections are apt to be injured by immersion in the nitric acid. In this case, after staining them in the fuchsin solution, they may be washed in distilled water, immersed in alcohol for a moment, and then placed in the following contrast stain for one to two hours: Distilled water, 100 cubic centimetres; saturated alcoholic solution of methylin-blue, 20 cubic centimetres; formic acid, 10 minims.

PROF. BAUMGARTEN'S METHOD OF DEMONSTRATING THE TUBERCLE-BACILLIS.

(From *Lancet*, July 15, 1882.)

For the demonstration of tubercle-bacilli in the sputum of phthisical patients Baumgarten recommends the following method as more convenient than those of Koch and Erhlich:—A little of the sputum is dried on the cover-glass, as recommended by the latter, and then treated with potash—one or two drops of a 33 per cent. solution of caustic potash added to a watch-glass of distilled water. The tubercle-bacilli can then be readily seen with a magnifying power of 400 or 500 diameters,

and a little pressure renders them still more distinct from the enclosing detritus of tissue. In order to preclude the possibility of confounding the bacilli of tubercle with those of other species, the cover-glass may be raised and placed aside until the layer of fluid on its under surface is dry, and then passed two or three times through a gas flame, and then on it may be placed a drop of an ordinary watery solution of aniline-violet or any other nucleus-tinting preparation of anilin. All the putrefactive bacteria then appear under the microscope as an intense blue or brown (according to the testing agent and its strength), while the tubercle-bacilli remain absolutely colourless, and can be seen with the same distinctness as in the ordinary potash preparation. The whole process does not occupy more than ten minutes.

Prof. Rindfleisch warms the staining solution by holding the watch-glass with the cover-glass floating on it over a flame until the fluid begins to steam; this shortens the process considerably, but the colour is apt to fade from the bacilli in a few hours.

THE AUTHOR'S METHOD.

(Published in the *Lancet*, August 5, 1882.)

The stain consists of—

Magenta . . .	grammes	2
Anilin oil (coml.) . .	c.c.	3
Alcohol, sp. gr. ·830 . .	c.c.	20
Aqua Dest. . . .	c.c.	20

To make the Stain.

Dissolve the anilin oil in the alcohol. Rub up the crystals in a glass mortar, with the alcohol, until they are

all dissolved; then add the distilled water while slowly stirring. The stain is now ready for use.

To use the Stain.

Spread a little sputum on a cover-glass and allow it to dry in the air protected from dust. When a specimen is wanted for diagnostic purposes at once, it may be dried over a small Bunsen, but for preparations that are to be kept for reference it is better to dry the sputum slowly in the air.

When thoroughly dry pass the cover-glass two or three times through the flame of a small Bunsen or spirit lamp. Pour a little of the Magenta solution into a watch-glass and place the cover-glass sputum downwards on it, allow it to remain for twenty or thirty minutes.

Make a dilute solution of commercial nitric acid, one part of acid to two of water, *which must be distilled.* Remove the cover-glass from the stain and wash in the nitric acid until all colour is removed, then wash in distilled water to remove all trace of acid, some of the colour will return, but if this does not become very deep it will not matter, if it does, wash again in the nitric acid. Make a saturated solution of Methylene blue, Methyl green or Iodine green in water, pour a little into a watch-glass and invert the cover-glass on it as before. Allow it to remain for five minutes, then wash in distilled water until no more colour comes away, drain off the water and place in a watch-glass with a little absolute alcohol for five minutes, drain off the alcohol and allow it to dry. When dry place a drop of Canada balsam solution on the centre of the cover-glass, and lower gently on to the slide, press lightly with a needle.

The above-mentioned stains are best for contrasts in

diagnostic work; but a watery solution of Chrysoidin is the best when the structure of the bacillus has to be studied.

The sputum used should be that coughed up by the patient the first thing in the morning, as later on it may only come from the back of the throat.

Heating the stain and drying off the alcohol over a flame will facilitate the process, but the result will not be so good.

The sputum should be spread as evenly as possible, and not too thickly.

When a specimen has been carefully made after the above method it will not fade, and the tubercle-bacilli will show out as bright red rods, while the pus cells are stained blue, green, or brown, according to the contrast stain used.

High powers are not required to see these bacilli: for ordinary clinical work, a $\frac{1}{2}$ or $\frac{4}{10}$ is enough to verify their presence. Beck's $\frac{1}{4}$, costing £1, will show them very well.

TO DOUBLE STAIN THE SPUTUM AT ONCE.

(Published by the Author, *Lancet*, May 5, 1883.)

This process does away with the use of nitric acid and is a very rapid one for diagnostic purposes, as it can be accomplished in five minutes after the sputum is dried on the cover glass.

The stain is made as follows: Take of rosanilin hydrochloride two grammes, methyl blue one gramme; rub them up in a glass mortar. Then dissolve anilin oil 3 c.c. in rectified spirit 15 c.c.; add the spirit slowly to the stains until all is dissolved, then slowly add distilled

water 15 c.c.; keep in a stoppered bottle. To use the stain: The sputum having been dried on the cover-glass in the usual manner, a few drops of the stain are poured into a test tube and warmed; as soon as steam rises pour into a watch-glass, and place the cover-glass on the stain. Allow it to remain for four or five minutes, then wash in methylated spirit until no more colour comes away; drain thoroughly and dry, either in the air or over a spirit lamp. Mount in Canada balsam.

This process gives the most satisfactory results, and the horrible nuisance of the nitric acid is avoided. It brings out the bacilli quite as well as the other process, and it stains all putrefactive bacteria and micrococci very deeply, so that in one field of the microscope blue micrococci and bacteria may be compared with the red bacilli of tubercle. The stain can be used cold equally well. The cover-glass in that case must be left in the stain for at least half an hour.

TO DEMONSTRATE THE TUBERCLE-BACILLUS IN SECTIONS OF HARDENED TISSUE.

Place the sections in the stain and allow them to remain for at least half an hour, then remove them to the dilute nitric acid and leave them in it until all the colour has been removed. Wash well in distilled water, and then place them in the methyl blue solution, where they must remain until they are deeply stained. Wash in distilled water until all superfluous colour is removed, and then transfer them to methylated spirit. Care must be taken that they do not curl up. They are then placed in absolute alcohol for five minutes. The alcohol is then drained off and they are placed in oil of cloves and mounted in Canada balsam solution.

It is a very difficult matter to get sections to lie flat on the slides after they have been subjected to the nitric acid, and here the double stain will be found invaluable, as the sections have only to be washed in spirit, as after any ordinary stain. They must remain in the stain for some time, the precise length of which has not yet been determined; the stain may be warmed and then the process will be facilitated, but there is a tendency to fade afterwards wherever these stains are warmed.

They must not be washed in water before spirit, but must be removed directly from the stain into methylated spirit, there they can be left until the excess of stain has washed off, when they must be placed in clean spirit and washed until the colour does not come out.

In this way very good specimens may be made with little difficulty. Sections, when properly stained by either of the above methods, may be kept for some time in ordinary spirit, but not in oil of cloves, which removes the colour from the bacilli.

ON THE DISCOVERY OF BACILLI IN THE CONDENSED AQUEOUS VAPOUR OF THE BREATH OF PERSONS AFFECTED WITH PHTHISIS.

(By Arthur Ransome, M.A., M.D. From the *Proceedings of the Royal Society*, No. 222, 1882.)

In the year 1869 I communicated to the Literary and Philosophical Society of Manchester a paper "On the Organic Matter of the Human Breath in Health and Disease" (Memoirs, vol. iv. 3rd Series, p. 234).

The method employed was to condense the vapour of the breath in a large glass globe, surrounded by ice and salt;

and the fluid so collected was then examined chemically and microscopically. The vapour in condensing was found to carry with it all the organic matter contained in the breath. Certain chemical variations in this fluid were noted, and in addition to epithelial scales, which were also found in health, the breath of diseased persons was found to contain certain organized bodies.

It appeared probable that the breath of persons in advanced stages of phthisis would contain the bacillus of tubercle, and that this organism could be rendered visible by the method of staining.

The aqueous vapour of the breath of certain cases of advanced phthisis was accordingly condensed in the above-mentioned manner, and each specimen was separately examined. In order to carry down the organic particles, and to afford a basis by which the substances obtained could be made to adhere to the microscopic cover-glasses, it was necessary to add some glutinous material to the condensed fluids. In some instances I used for this purpose fresh white of an egg, in others mucus from the mouth, that had been separately examined by staining, and which had been found free from bacilli. No attempt was made to sterilize any of the fluids, the ordinary bacteria of putrefaction being left unstained in the process used.

The method of staining employed was that suggested by Dr. Heneage Gibbes, in which magenta and aniline are first used, and then after discharging the colour, from all but the bacilli, by dilute nitric acid, chrysoidin is used to throw them into relief. (See *Lancet*, August 5, 1882.)

I have now to state that in the aqueous vapour obtained from two persons suffering from phthisis, I have found specimens of a bacillus, which takes the

staining in the same manner as the bacillus found in phthisical sputa and in tubercle, and which is indistinguishable from that organism. In several cases of acute phthisis the search for the organism was unsuccessful, and none were found in the aqueous vapour condensed from the waiting room of the Consumption Hospital in Manchester.

Koch has shown that the dust from dried phthisical sputa is capable of conveying the disease, but the above-mentioned discovery of the bacillus in the breath renders it probable that particles contagious to susceptible individuals are constantly being breathed in with the air, and it is possible that at some future time, the bacillus of tubercle may, by careful cultivation of the vapour of crowded rooms, be obtained from this source.

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